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# QUALITY ASSURANCE GUIDELINES FOR ENVIRONMENTAL HEALTH RESEARCH

by

P. A. Cunningham
K. W. Gold
T. J. Hughes
L. E. Myers
C. E. Tatsch

EPA Contract No. 68-02-3226

EPA Project Officer: Ferris B. Benson
RTI Project Leader: C. E. Tatsch

Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

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16. ABSTRACT

This document provides conceptual guidelines for the development, implementation and evaluation of research task quality assurance plans for the staff of the Health Effects Research Laboratory (HERL/RTP) of the U.S. Environmental Protection Agency, Research Triangle Park, North Carolina. It is designed to assist project officers in applying quality assurance concepts to each phase of a research task, from the initial planning through final report preparation. It is designed to assist management staff in evaluating these plans in the implementation of Agency QA policy.

The guidelines describe the HERL-RTP QA organization and the QA responsibility of both management and technical research personnel in relation to the mandatory Agency QA policy and task data quality requirements. Aspects of research tasks that must be considered by project officers in the development or review of QA elements are treated in detail. Specific guidelines for atmosphere generation, dose monitoring and animal research are also included.

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# FOREWORD

The U.S. Environmental Protection Agency's Health Effects Research Laboratory located at Research Triangle Park, North Carolina, conducts an extensive research program to evaluate the human health implications of environmental factors related to contemporary society. The purpose of this research is to develop appropriate information for formulating sound environmental policies for protecting and improving public health and welfare while enhancing the nation's productivity. To this end, the Laboratory conducts a comprehensive environmental research program in toxicology, epidemiology, and research on human subjects under controlled laboratory conditions.

The quality of the data resulting from this research is an overriding factor in determining their usefulness to EPA. In recognition of the importance of data quality assurance, our Laboratory instituted an active, comprehensive program to coordinate the development and implementation of effective quality assurance planning into all research within the Laboratory. More recently, the Administrator has required quality assurance for all environmentally related measurement activities supported by the Agency. This substantially enhances the quality assurance aspects of our own research measurements.

This document represents the current statement of our effort. I am confident that full implementation of our data quality assurance policy, with the help of the guideline manuals and the increased application of quality assurance principles, will enhance the scientific merit of our research program.

F. Gordon Hueter, Director
Health Effects Research Laboratory

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# SECTION 1

## ABSTRACT

This document is a statement of the Quality Assurance (QA) policy of the Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina (HERL-RTP). It describes the HERL-RTP QA organization and the QA responsibilities of both management and technical research personnel in relation to the mandatory Agency QA policy and task data quality requirements. It provides guidelines for managers in the implementation of Agency QA policy and evaluation of research documentation, and presents guidelines for project officers for (1) development of QA Project Plans for intramural research and support tasks, (2) preparation of requests for proposals, (3) preaward QA evaluation of proposals, and (4) review and evaluation of QA Program or Project Plans for extramural tasks. Aspects of research tasks that must be considered by project officers in the development or review of QA elements are treated in detail. Specific guidelines for atmosphere generation, dose monitoring, and animal research are also included. These guidelines are reviewed and revised annually by the HERL-RTP QA officer.

# SECTION 2
# INTRODUCTION

## 2.1 LABORATORY OBJECTIVES

The Health Effects Research Laboratory, Research Triangle Park, North Carolina (HERL-RTP), conducts animal and human studies under controlled conditions and performs studies on human populations to assess the health hazards of environmental pollutants. The effects of environmental pollutants, both singly and in combination, are investigated; pollutants studied include air pollutants, pesticides, toxic substances, and nonionizing radiation. Controlled laboratory studies are conducted to determine effects of pollutants on normal biological function as measured by clinical, chemical, biochemical, physiological, histopathological, growth, reproduction, and other parameters. In addition, HERL-RTP develops, evaluates, and improves analytical chemical methods and biological screening techniques for direct and indirect measurement of exposure to environmental toxicants. It also serves as a resource for information on the health effects of environmental pollutants and coordinates health-related programs with international organizations.

## 2.2 BACKGROUND

Because of an increased awareness of the serious health effects of environmental pollutants and of the need for adequate data quality to support risk assessment and control strategies, HERL-RTP management initiated a formal Laboratory-wide data quality program in May 1976 with the issuance of a "Quality Assurance Plan."[1] Subsequently, a Quality Assurance Officer was appointed as chairman of the Quality Assurance Committee for the purpose of designing and implementing a Laboratory-wide QA Program. Quality assurance guidelines have since been developed for management policy,[2] research task planning,[3] and environmental pollutant measurements.[4] [5] [6] Guidelines for quality assurance in selected areas of biological research are currently being developed to complete the Quality Assurance Program at HERL-RTP.

The HERL-RTP QA Program is further supported by EPA's recent commitment to a mandatory Agency-wide QA Program. Agency policy initiated by the Administrator in memoranda of May 30, 1979,[7] and June 14, 1979,[8] requires participation in a centrally managed Quality Assurance Program of all EPA Laboratories, Program Offices, Regional Offices, and those monitoring and measurement efforts supported or mandated through contracts, regulations, or other formalized agreements.

The Office of Research and Development (ORD) is responsible for developing, coordinating, and directing implementation of the Agency QA Program. ORD has delegated this responsibility to the Quality Assurance Management Staff (QAMS) of the Office of Monitoring Systems and Technical Support.

To implement Agency policy, EPA Laboratories, Program Offices, and Regional Offices are required to prepare QA Program Plans covering all intramural and extramural monitoring and measurement activities that generate and process environmentally related data for Agency use. A QA Program Plan for HERL-RTP has been developed and submitted to QAMS for approval.

In addition, every project officer is required to prepare a comprehensive Quality Assurance Project Plan for each project under his control, in accordance with Agency criteria and guidelines.[3] [9] This document presents guidelines for Laboratory managers and project officers for developing QA Project Plans.

## 2.3 DEFINITIONS

The American Society for Quality Control has carefully defined terms that apply to quality.[10] The Quality Assurance Handbook for Air Pollution Measurement Systems[4] provides similar definitions of quality terminology applicable to air pollution data collection systems. Several terms related specifically to health research data quality are defined below as they are used in these guidelines.

### 2.3.1 Quality

> Quality is the totality of characteristics of research data that bear on their ability to satisfy previously specified criteria. For laboratory measurement systems, accuracy, precision, and representativeness are of major importance. Completeness is appropriately applied to larger systems, such as air monitoring networks.

### 2.3.2 Quality Assurance

Quality assurance is a program of planned, systematic actions that are necessary to ensure that specified data quality criteria are achieved. QA planning is necessary at the management level in the development of QA policy, and at the project level in the development of research protocols by project officers (see Section 2.3.6). QA activities consist of: (1) quantitative measurements, such as interlaboratory tests or performance audits; and (2) qualitative measures, such as site visits or systems audits, to evaluate the capability of a total measurement system for providing specified quality data. Quality assurance, in planning and execution, is a management function independent of task operating personnel.

### 2.3.3 Quality Control

Quality control (QC) is a system of activities designed to achieve and maintain a previously specified level of quality in data collection, processing, and reporting. Quality control is performed by the task or project personnel. QC activities include control or correction for all variables affecting data quality (see Section 4.5).

### 2.3.4 Environmental Measurement

The term "environmental measurement" applies to all field and laboratory investigations that generate data involving the measurement of chemical, physical, or biological parameters in the environment, such as determining the presence or absence of priority pollutants in waste streams; health and ecological effect studies; clinical and epidemiological investigations; engineering and process evaluations; studies involving laboratory simulation of environmental events; and studies or measurements on pollutant transport and fate, including diffusion models.

### 2.3.5 Task

A task is any project, intramural or extramural, that produces or uses environmentally related data.

### 2.3.6 Protocol

The term protocol includes all task or project planning documents. Specifically included are research plans, support activity procedure statements, contractors' work plans, scopes-of-work, and plans for total task quality assurance, i.e., QA Project Plans.

### 2.3.7 QA Program Plan

A QA Program Plan is a written document that presents in general terms the overall policies, organization, objectives, functional responsibilities (within the organization), etc., designed to achieve specified data quality goals of a particular organization (e.g., EPA Laboratory, Program Office, Regional Office, contracting organization).

### 2.3.8 QA Project Plan

A QA Project Plan is a written document that details the policies, organization, objectives, functional activities, and specific QA and QC activities designed to achieve data quality goals or requirements of a specific project. The QA Project Plan describes procedures to be used to routinely assess data precision, accuracy, completeness, representativeness, and comparability of the measurements involved.

### 2.3.9 Project Officer

The project officer is that individual who is assigned overall responsibility for a project from inception through completion. This responsibility covers both technical and QA aspects of the project.

### 2.3.10 QA Officer

The QA officer is that individual who is assigned the responsibility for overview and guidance of the QA program for an organization or for a specific project. The QA officer should be in a position to provide independent and objective evaluations and assessments of the effectiveness of the QA program and to provide timely feedback and recommendations.

### 2.3.11 Contracting Officer

The contracting officer is that individual who is assigned the responsibilty for ensuring that contracting is done as authorized by law and regulation.

### 2.3.12 QA Performance Audit

A QA performance audit is a quantitative analysis or check with a material or device with known properties or characteristics to determine the accuracy of a measurement system. Performance audits may require either the identification or the quantitation of specific elements or compounds or both.

### 2.3.13 QA Systems Audit

A QA systems audit consists of a systematic onsite qualitative review of facilities, equipment, training, procedures, recordkeeping, data validation, data management, and reporting aspects of the total measurement system. This may be required to assess the capability of a measurement system to generate data of the required quality or to determine compliance of a project with specified QA requirements.

## 2.4 REFERENCES

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# SECTION 3

# MANAGEMENT POLICY

This section describes HERL-RTP management policies and goals and the QA organization responsible for development and implementation of the Laboratory Quality Assurance Program.

All QA activities within HERL-RTP will be carried out in accordance with Agency mandates and guidance specified by the Quality Assurance Management Staff (QAMS) of the Office of Research and Development (ORD). Planning for the incorporation of suitable QA measures into measurement activities is the responsibility of project officers. It is the responsibility of management to ensure that all project-related documents or plans incorporate adequate QA measures. It is also management's responsibility to ensure that QA Project Plans are implemented and that project data are of adequate and documented quality. The HERL-RTP QA organization, consisting of a Quality Assurance Committee chaired by the QA officer, is available to all Laboratory technical and management personnel for consultation or active participation in development and review of QA Project and Program Plans.

## 3.1 QUALITY ASSURANCE PROGRAM GOALS

The goal of the HERL-RTP Quality Assurance Program is to ensure, assess, and document the quality of laboratory and field data used by EPA in assessing the human health effects of pollutants and in developing adequate control strategies.

Specific objectives of the HERL-RTP QA Program are to:

1. Establish a QA organization within HERL-RTP having responsibility and authority for developing and implementing a Laboratory QA Program.

2. Develop a program to familiarize all HERL-RTP personnel with the basic concepts of quality assurance.

3. Establish and maintain written QA guidelines to assist HERL-RTP personnel in the logical development of general and specific QA plans for current and future HERL-RTP research.

4. Provide criteria for evaluating proposed and ongoing tasks for appropriateness to the data quality requirements of HERL-RTP.

5. Ensure that all data reported include data quality estimates of representativeness, precision, accuracy, comparability, and, when appropriate, completeness.

6. Implement procedures to review quality aspects of data currently being collected or data collected in the past, as deemed appropriate.

7. Encourage the use and development of methods of analysis and data treatment that are capable of meeting the data quality requirements of HERL-RTP.

8. Develop and implement a comprehensive Laboratory QA audit program covering all routine measurement methods in use.

9. Monitor the operational performance of HERL-RTP through appropriate intralaboratory and interlaboratory quality evaluation programs.

10. Ensure that protocols with approved QA Project Plans are developed and implemented by HERL-RTP investigators and contractors.

11. Develop mechanisms for identifying data quality problem areas, alerting management to them, and evaluating the proposed solutions to such problems.

## 3.2 QUALITY POLICIES

It is the policy of HERL-RTP that the program of quality assurance and quality control will be appropriate to ensure that all data collected are of known and documented quality. QA Program requirements cover all activities supported or required by HERL-RTP that generate environmentally related measurement data.

### 3.2.1 Scope of the Laboratory QA Program

The HERL-RTP QA Program covers all funded tasks--intramural and extramural, contract, cooperative, and interagency agreement; it requires that quality control considerations be included in all requests for proposals (RFP's), research proposals and evaluations, work plan approvals, project plans, and project reports. All measurement activities planned or conducted within HERL-RTP must have a QA Project Plan incorporated into the research protocol and approved by the QA officer.[1] Specifically, the QA Project Plan ensures that:

1. The level of data quality needed will be determined and stated before the data collection effort begins.
2. All data generated and reported will be of the quality and integrity established by each QA Project Plan.

### 3.2.2 Quality Assurance Project Plan Design

Project officers are responsible for the design of QA Project Plans for intramural tasks and for incorporation of these plans into research protocols. QA Project Plans for tasks conducted under cooperative or interagency agreement or contract are called for in the RFP and must be prepared by the cooperator or contractor.

QA Project Plans must include all QA/QC activities appropriate to the project data quality requirements and to the methods of data collection and data processing employed. The following aspects of project data quality should be addressed in all QA Project Plans and are individually discussed in detail in Section 4 of this document:

- Experimental Design
- Personnel
- Facilities
- Services
- Equipment
- Supplies
- Recordkeeping
- Chain of Custody
- Sample Collection
- Sample Analysis
- Internal Audits
- Preventive Maintenance
- Calibration
- Documentation Control
- Configuration Control
- Data Validation
- Feedback and Corrective Action
- Data Processing and Analysis
- Report Design

### 3.2.3 Quality Assurance Project Plan Review and Approval

All QA Project Plans must be reviewed and approved by appropriate HERL-RTP management prior to project funding. Specifically, approval by the section chief, branch chief, division director, and QA officer is required. Additionally, the project officer must review and approve all QA Project Plans for extramural tasks under his supervision. Periodic review of QA Project Plans by the project officer and the QA officer throughout the project term provides the means by which management may assess if suitable data quality has been obtained in a cost-effective manner. Assistance from the QA organization is available to management for the evaluation of QA Project Plans and the effectiveness of their implementation.

Systems and performance audits and interlaboratory/interfield comparison studies will be conducted on measurement projects within HERL-RTP as planned by the QA officer and the appropriate division director to assess the adherence to, and adequacy of, the approved QA Project Plans and to assess the need for corrective action.

## 3.3 QUALITY ASSURANCE PROGRAM ORGANIZATION

In planning QA programs for particular tasks, project officers should attempt to identify all variables that may affect the quality of data to be produced and include appropriate QA/QC measures in QA Project Plans. As a result of an increasing awareness of the need for quality assurance in Agency programs, EPA has developed comprehensive QA guidelines.[2-5] In addition, Federal standards for nonclinical laboratories have been promulgated,[6] health effects test standards have been proposed for testing under the Toxic Substances Control Act,[7] [8] and guidelines for quality assurance practices in health laboratories have been published by the American Public Health Association.[9] Project officers should refer to these, when appropriate, in developing QA Project Plans.

To support project officers and management in the development and implementation of QA Project Plans, the quality assurance organization is interwoven with the existing HERL-RTP management. The structure of the QA organization, the functional responsibility of QA personnel, and the lines of communication for achievement of a cost-effective QA Program are discussed below.

### 3.3.1 Organizational Structure for Quality Assurance

The HERL-RTP management structure is shown in Figure 3-1. All QA management responsibilities are assigned to a Quality Assurance officer. The independence and objectivity of the QA Program is supported by the QA officer's organizational independence of all divisions involved in the data generation process. The QA officer reports directly to the HERL-RTP Laboratory Director on QA matters and is primarily responsible for the design and implementation of the Laboratory QA Program. The QA Committee, chaired by the QA officer, is responsible for evaluating the effectiveness of the Program throughout the Laboratory and for recommending improvements. The QA Committee members act as liaison between the QA Committee and their divisions or offices.

All divisions within HERL-RTP (shown in Figure 3-1) are covered by QA Program requirements. Interfacing of the QA organization with various levels of management on QA matters is shown in Figure 3-2.

### 3.3.2 Assignment of Responsibilities

Although each person involved in the generation of data is implicitly a part of the QA Program, certain individuals have specific, assigned QA responsibilities. Refer to Figure 3-2 in the following discussion.

#### 3.3.2.1 HERL-RTP Laboratory Director--

The HERL-RTP Laboratory Director has overall responsibility for all Laboratory activities, including quality assurance. Because the success of the QA Program ultimately depends on the Director's full support of QA management, it is his/her responsibility to enlist and encourage the cooperation of all HERL-RTP personnel in the program.

#### 3.3.2.2. Quality Assurance Officer--

The QA officer has primary responsibility for all Laboratory QA activities and reports directly to the HERL-RTP Laboratory Director. His/her responsibilities include the development, evaluation, and documentation of QA policy and procedures appropriate to the Laboratory objectives. This includes evaluation of the cost-effectiveness of QA programs and plans and recommendations for their improvement.

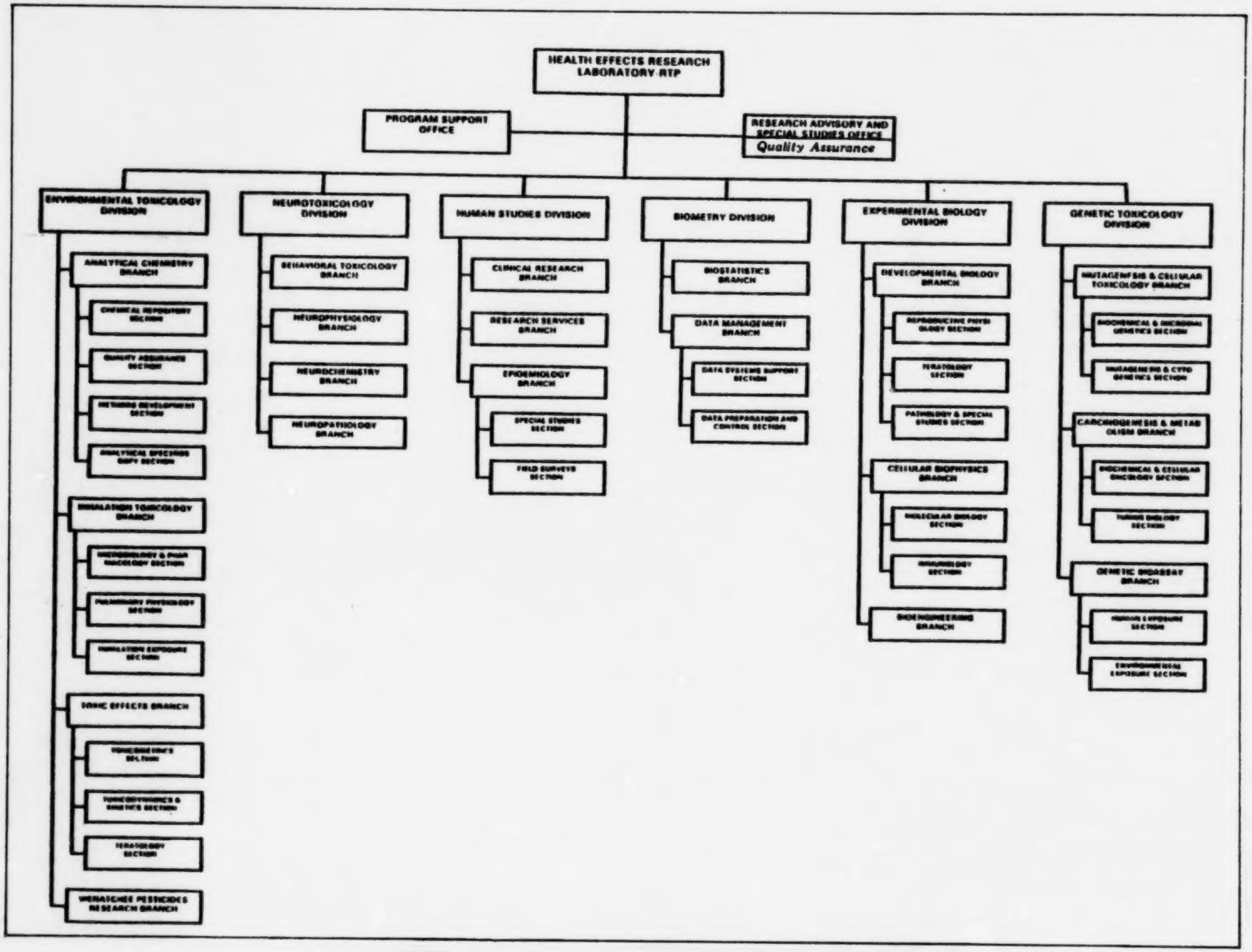

HEALTH EFFECTS RESEARCH LABORATORY-RTP
PROGRAM SUPPORT OFFICE
RESEARCH ADVISORY AND SPECIAL STUDIES OFFICE
Quality Assurance
ENVIRONMENTAL TOXICOLOGY DIVISION
ANALYTICAL CHEMISTRY BRANCH
CHEMICAL REPOSITORY SECTION
QUALITY ASSURANCE SECTION
METHODS DEVELOPMENT SECTION
ANALYTICAL SPECTROSCOPY SECTION
INHALATION TOXICOLOGY BRANCH
MICROBIOLOGY & PHARMACOLOGY SECTION
PULMONARY PHYSIOLOGY SECTION
INHALATION EXPOSURE SECTION
TOXIC EFFECTS BRANCH
TOXICOMETRICS SECTION
TOXICODYNAMICS & KINETICS SECTION
TERATOLOGY SECTION
WENATCHEE PESTICIDES RESEARCH BRANCH
NEUROTOXICOLOGY DIVISION
BEHAVIORAL TOXICOLOGY BRANCH
NEUROPHYSIOLOGY BRANCH
NEUROCHEMISTRY BRANCH
NEUROPATHOLOGY BRANCH
HUMAN STUDIES DIVISION
CLINICAL RESEARCH BRANCH
RESEARCH SERVICES BRANCH
EPIDEMIOLOGY BRANCH
SPECIAL STUDIES SECTION
FIELD SURVEYS SECTION
BIOMETRY DIVISION
BIOSTATISTICS BRANCH
DATA MANAGEMENT BRANCH
DATA SYSTEMS SUPPORT SECTION
DATA PREPARATION AND CONTROL SECTION
EXPERIMENTAL BIOLOGY DIVISION
DEVELOPMENTAL BIOLOGY BRANCH
REPRODUCTIVE PHYSIOLOGY SECTION
TERATOLOGY SECTION
PATHOLOGY & SPECIAL STUDIES SECTION
CELLULAR BIOPHYSICS BRANCH
MOLECULAR BIOLOGY SECTION
IMMUNOLOGY SECTION
BIOENGINEERING BRANCH
GENETIC TOXICOLOGY DIVISION
MUTAGENESIS & CELLULAR TOXICOLOGY BRANCH
BIOCHEMICAL & MICROBIAL GENETICS SECTION
MUTAGENESIS & CYTOGENETICS SECTION
CARCINOGENESIS & METABOLISM BRANCH
BIOCHEMICAL & CELLULAR ONCOLOGY SECTION
TUMOR BIOLOGY SECTION
GENETIC BIOASSAY BRANCH
HUMAN EXPOSURE SECTION
ENVIRONMENTAL EXPOSURE SECTION

Figure 3-1. Functional management structure, HERL-RTP.

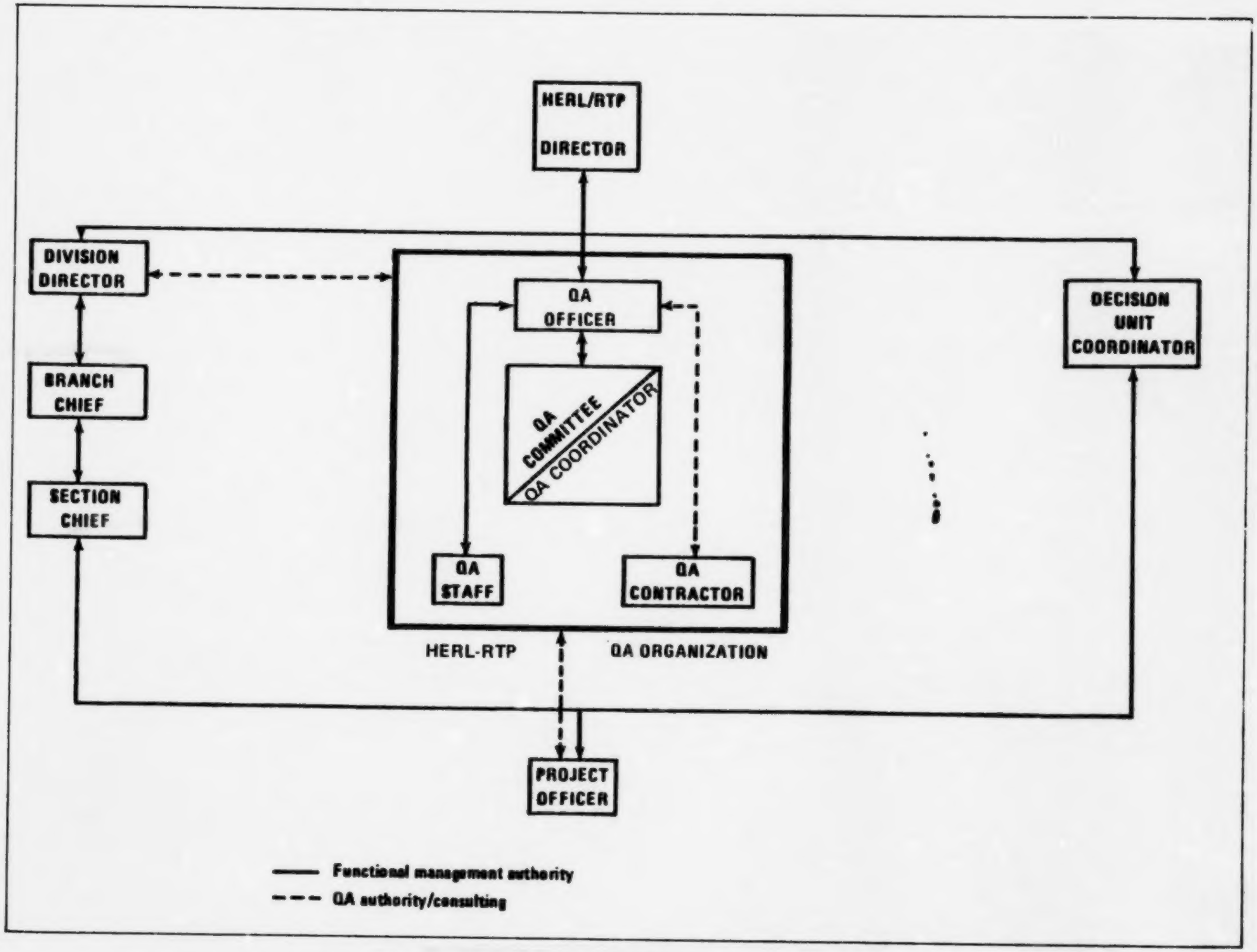

HERL/RTP DIRECTOR
DIVISION DIRECTOR
BRANCH CHIEF
SECTION CHIEF
QA OFFICER
QA COMMITTEE
QA COORDINATOR
QA STAFF
QA CONTRACTOR
HERL-RTP
QA ORGANIZATION
DECISION UNIT COORDINATOR
PROJECT OFFICER
Functional management authority
QA authority/consulting

Figure 3-2. Interactions of the QA organization with other HERL-RTP management.

As advisor to the Laboratory Director, the QA officer regularly reports on the status of the Laboratory QA Program, identifies specific needs (e.g., methods development and problem areas), and recommends specific courses of action for strengthening the Program.

As chairman of the QA Committee, the QA officer initiates development of Laboratory-wide QA guidelines and procedures. He/she coordinates methods development efforts for new QA procedures for specific HERL-RTP research techniques, and assesses data provided by the Committee regarding evaluation of the QA Program. He/she is also responsible for the development of special audit programs for Laboratory-wide measurement techniques.

As QA consultant, he/she is available to consult with and recommend to the HERL-RTP professional staff (project officers, investigators, etc.) appropriate and necessary QA methods and plans for ensuring the quality of the research data produced.

Motivation of personnel is a critical factor in the success of the Laboratory QA Program. Hence, a major responsibility of the QA officer is to ensure that all personnel have a good understanding of the Laboratory QA Program, an understanding of their responsibilities, and an appreciation of the importance of their roles to the overall success of the program.

#### 3.3.2.3 Quality Assurance Coordinator/Committee--

Each Division Director designates QA coordinators to serve on the HERL-RTP QA Committee. The QA coordinators recommend and review proposals for improvements in QA policies and procedures, and report and evaluate potential data quality problem areas. Within their respective divisions, the QA coordinators consult on matters of quality assurance, serving as a primary source of information on research quality assurance matters, reviewing contract proposals for QA aspects, and helping to implement the Laboratory QA Program.

The QA Committee serves as an advisory committee to the Laboratory Director. Specifically, the Committee's functions include assisting in the evaluation and refinement of data quality objectives of the QA Program to ensure that they meet the Laboratory needs with minimum disruption of existing workloads and procedures, reviewing recommendations presented to the Committee, and assessing the effectiveness of the QA Program.

### 3.3.2.4 Quality Assurance Staff--

The QA staff is composed of technical personnel; key members hold joint appointments in other research areas at HERL-RTP. They are responsible for providing the necessary technical expertise to ensure adequate implementation and review of the Laboratory QA Program. In particular, they are available to consult with project officers and management on the technical aspects of specific task activities that affect overall data quality (e.g., methods development) and to aid the QA officer in the development, implementation, and evaluation of audit programs for both Laboratory-wide and task-specific measurement techniques.

### 3.3.2.5 Quality Assurance Contractors/Consultants--

External QA contractors/consultants assist in implementing major parts of the HERL-RTP QA Program. Their tasks may include development and/or evaluation of Laboratory QA guidelines and plans, development and implementation of Laboratory-wide audit programs, methods development, and provision of analytical services.

### 3.3.2.6 Decision Unit Coordinator--

Evaluation of the need for specific types of research is an essential part of the Laboratory QA Program. The Decision Unit (DU) Coordinator is the functional manager for each general program area, and, as such, is responsible for distributing resources (i.e., funding and manpower) and for evaluating the relevance of each proposal (and task) in his/her program area to the broadly defined Laboratory and Agency goals. In addition, he/she is responsible for identifying impending needs in specific areas of research and encouraging proposals for new tasks in these areas, thus aligning the production of research data with the overall Laboratory and Agency needs.

### 3.3.2.7 Project Officer--

As task manager, the project officer is responsible for fulfilling the technical and administrative requirements of each task. It is his/her responsibility to adequately ensure and document the quality of the task product, including both the research data and conclusions. The project officer draws upon his/her professional training and expertise, in collaboration with the HERL-RTP QA organization, to determine which QA/QC techniques most appropriately apply to a particular task and to develop task-specific QA Project Plans.

To ensure adequate data quality, the project officer should anticipate events that might threaten data quality (e.g., slowly degrading reagents), provide contingencies for anticipated failures and problems, and obtain objective evaluation of task data quality as the task progresses. These topics are discussed in detail in Section 4 of this document.

To adequately document QA plans and activities, the project officer must include data quality considerations in all task-related documents and collaborate with the HERL-RTP QA organization in external QA activities (see Section 5).

#### 3.3.2.8 Functional Managers--

Functional managers (e.g., division director, branch chief, section chief) are responsible for ensuring the quality of research data produced under their direction. This responsibility includes review and approval of QA Project Plans and periodic evaluation of ongoing QA programs. Peer review of QA techniques by the QA Committee may also be requested by management for evaluation of task planning and execution. Critical aspects of task data quality addressed in Section 4 of this document should be referred to for guidance in evaluating proposals, plans, progress reports, and final reports.

Management support of QA programs should be visible and active. Such support may include:

1. Development and support of data evaluation techniques appropriate to health effects research, coordinated through the QA organization if appropriate.
2. Provision of information to project officers on interlaboratory and intralaboratory testing programs and encouragement of their participation in them.
3. Development of QA programs and techniques for health effects research, using available standards and procedures.
4. Development of standards and QA procedures for new measurement methods.

Such activities will demonstrate the commitment of HERL-RTP management to the Quality Assurance Program.

## 3.4 REFERENCES

1. U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Protocols, Research Triangle Park, NC, Director's Memorandum, September 25, 1979.

2. U.S. Environmental Protection Agency, Health Effects Research Laboratory, Guides for Quality Assurance in Environmental Health Research, EPA-600/1-79-013, Research Triangle Park, NC, 1979.

3. U.S. Environmental Protection Agency, Health Effects Research Laboratory, Development of Quality Assurance Plans for Research Tasks, EPA-600/1-78-012, Research Triangle Park, NC, 1978.

4. U.S. Environmental Protection Agency, Quality Assurance Research Plan, FY 1978-81, EPA-600/8-77-008, Washington, DC, July 1977.

5. U.S. Environmental Protection Agency, Quality Assurance Guidelines for Biological Testing, EPA-600/4-78-043, Las Vegas, NV, August 1978.

6. Non-Clinical Laboratories Studies: Regulations for Good Laboratory Practice, Federal Register, December 22, 1978, pp. 59985-60025.

7. Proposed Health Effects Test Standards for Toxic Substances Control Act Test Rules, Federal Register, May 9, 1979, p. 27334.

8. Good Laboratory Practice Standards for Health Effects, Federal Register, May 9, 1979, p. 26362.

9. Inhorn, S. L., ed., Quality Assurance Practices in Health Laboratories, American Public Health Association, 1977.

## SECTION 4

## GUIDELINES FOR PROJECT OFFICERS

By memoranda of May 30, 1979,[1] and June 14, 1979,[2] the EPA Administrator established the goal of the Agency Quality Assurance (QA) Program to ensure that all environmentally related measurements funded or mandated by EPA and interagency agreements be scientifically valid, defensible, and of known precision and accuracy. As part of the mandatory Agency QA Program, HERL-RTP requires that protocols for all projects producing or using environmentally related data contain approved QA Project Plans.

This section presents guidelines for project officers for (1) development of QA Project Plans for intramural research and support tasks, (2) preparation of requests for proposals (RFPs), (3) preaward QA evaluation of proposals, and (4) review and evaluation of QA Program or Project Plans for extramural tasks. A detailed checklist of specific items that should be considered in all QA planning or review of task-related documents is given in Figure 4-1. The relative impact of each of these aspects on overall data quality will vary between tasks; however, no element should ever be deleted from consideration.

In the following discussion, as elsewhere in this document, the terms "must" and "should" are used with very specific and distinct meanings: "must" is used where there is an actual requirement as stated by the QAMS or HERL-RTP management; "should" is used to denote a recommended or desirable activity. It is understood that specific requirements apply to all HERL-RTP projects; recommendations apply only to those projects or activities where appropriate.

Aspects of research tasks that should be considered by the project officer in the development or review of QA elements are detailed in Sections 4.1 through 4.5 under the following general areas:

1. General approach to quality control in research.

## Project Officer QA/QC Checklist

QA/QC Item

### Identification

Comments

☐ Title page with provision for approval and review signatures
☐ Table of contents

### Project Description

☐ Project organization and responsibility
☐ Objectives
☐ Hypotheses
☐ Experimental design
☐ QA objectives for measurement data

### Personnel

☐ Training and experience
☐ Adequacy (numbers)
☐ Résumés of key task personnel

### Facilities

☐ Appropriateness to task requirements
☐ Environmental aspects (e.g., temperature, lighting, ventilation)
☐ Maintenance (preventive and corrective)
☐ Inspection procedures
☐ Configuration control
☐ Security
☐ Safety provisions
☐ Support services

### Equipment

☐ Appropriateness to task requirements
☐ Maintenance (preventive and corrective)
☐ Configuration control
☐ Safety provisions
☐ Recordkeeping (i.e., documentation of calibration and maintenance history)

### Supplies

☐ Certification
☐ Acceptance screening

Figure 4-1. Project officer QA/QC checklist for planning and evaluating QA project plans.

( ) Animal care and testing procedures
( ) Storage
( ) Stockroom tracking system
( ) **Recordkeeping**
( ) **Chain-of-Custody**

### Sampling Procedures

( ) Sample collection
( ) Sample analyses

### Data Management

( ) Collection
( ) Storage and backup
( ) Transfers
( ) Reduction
( ) Software
( ) Analysis
( ) Reporting

### Quality Control/Quality Assurance

( ) Specific procedures to be used to routinely assess data precision, accuracy, and completeness
( ) Internal audits
( ) External audits
( ) Calibration
( ) Documentation control
( ) Configuration control
( ) Data validation
( ) Feedback and corrective action
( ) Quality assurance reports to management

### Special Requirements

( ) Radiation safety form
( ) Toxic chemical form
( ) Request for Animal Procurement and Care

(√) Satisfactory
(U) Unsatisfactory
(NA) Not Applicable

NOTE: Specific checklists for animal facilities, animal husbandry, and animal testing should be completed, if applicable (see Section 7).

Figure 4-1 (continued)

2. Planning--experimental design, personnel, facilities, equipment, supplies, recordkeeping, and chain of custody.
3. Experimental--sample collection and analysis.
4. Data quality control activities--internal audits, preventive maintenance, calibration, documentation control, configuration control, data validation, feedback, and corrective actions.
5. Data processing, analysis, and reporting.

## 4.1 GENERAL APPROACH

The purpose of this section is to assist project officers in the uniform implementation of QA requirements for intramural tasks, cooperative agreements, contracts, and interagency agreements. Project officers should work closely with the HERL-RTP QA officer on all QA matters.

### 4.1.1 Intramural Tasks

#### 4.1.1.1 Research Tasks--

The project officer is responsible for preparation of QA Project Plans and inclusion of these plans in the research protocol for each intramural research task. To assist project officers in this effort, the HERL-RTP QA organization has developed guidelines for preparation of intramural research protocols (see Appendix A). These guidelines contain specific instructions for preparing QA Project Plans and should be consulted. In particular, it should be noted that all intramural support tasks that may be involved in the proposed research task must be referenced in the research protocol by task number and project officer and must have an approved protocol (including a QA Project Plan) on file with the QA officer prior to approval of the research protocol.

The QA officer may participate in the development of QA Project Plans at the request of the project officer and must approve all QA Project Plans prior to project funding. The project officer and the QA officer should also review and evaluate the implementation of QA Project Plans throughout the project term via periodic systems and/or performance audits and review of regular QA project reports. Upon completion of the project, the project officer and the QA officer should assess the actual performance of the planned QA activities and the subsequent results. The final QA project report must contain the results of this assessment and must be approved by the QA officer.

#### 4.1.1.2 Support Tasks--

The project officer for each HERL-RTP support task is responsible for development of an appropriate QA Project Plan and incorporation of this plan into the task protocol. To assist project officers in this effort, the HERL-RTP QA organization has developed specific guidelines for preparation of intramural support protocols including QA Project Plans (see Appendix A). In addition, project officers may consult with the QA officer, or his/her staff, in the preparation of QA Project Plans.

The QA officer must approve all intramural support task QA Project Plans prior to project funding. Since approval for funding of any intramural research task supported by an intramural support task is contingent on approval of the QA Project Plan for the support task, all intramural support tasks must have approved QA Project Plans on file with the QA officer.

### 4.1.2 Cooperative Agreements

The originating project officer must notify the QA officer of all cooperative agreements involving the generation or use of environmentally related measurement data during the planning phase. It is the responsibility of the QA officer to ensure that QA requirements are met prior to awarding the cooperative agreement.

Preparation of QA Project Plans for cooperative agreements is the responsibility of the cooperator. All cooperating institution QA Program and Project Plans must be consistent with EPA criteria and regulations. QA Program Plans must be included in the proposal and reviewed by the project officer and the QA officer for approval prior to funding. Detailed QA Project Plans may be required to be included in the original proposal or to be submitted within a specified time after award for approval by the QA officer. Where appropriate, final approval may be contingent upon successful completion of a systems audit of the offeror, as directed by the QA officer.

To facilitate systematic review of QA requirements for HERL-RTP projects covered by cooperative agreements, ORD QAMS is preparing Guidelines and Specifications for Implementing Quality Assurance Requirements for EPA Grants and

Cooperative Agreements Involving Environmental Measurements.[3] These should be consulted when available. At present, project officers should consult with the QA officer for specific guidance on QA requirements for cooperative agreements to ensure compliance with QAMS criteria. The QAMS guidelines and review forms for interagency agreements (reference 4, Appendix B) may be referred to for general guidance.

The project officer and the QA officer must review and evaluate the effectiveness of the QA Project Plan during the project and must assess the quality of data generated and processed. Upon completion of the project, the project officer and the QA officer must assess the overall effectiveness of the planned QA activities and the study results. The final QA project report must contain the results of this assessment and must be approved by the QA officer.

### 4.1.3 Contracts

QA requirements for HERL-RTP contracts must be consistent with existing EPA criteria as specified in the recently published QAMS Guidelines and Specifications for Implementing Quality Assurance Requirements for EPA Contracts and Interagency Agreements Involving Environmental Measurements.[4]

To assist project officers and QA officers in QA planning and decision-making for their projects (contracts), QAMS designed QA Form QAR-C (see Appendix B). Although project officers have responsibility for their projects and are the official contacts with the contracting officer, they should consult with and obtain agreement from the QA officer on all QA matters. It is the responsibility of the QA officer to ensure that QA requirements are met prior to the awarding of the contract or interagency agreement.

#### 4.1.3.1 QA Requirements in the Request for Proposal--

Prior to release of the purchase request to Procurement and Contracts Management Division, the project officer should determine if the project involves the generation of environmentally related measurement data. If so, the project officer must delineate in the RFP the QA requirements, approved by the QA officer, which each offeror must include in the proposal. The statement of work should state as clearly as possible: (1) the objectives of the project; (2) the data quality acceptance criteria, including minimum requirements for precision, accuracy, representativeness, completeness, and comparability; and (3) the quality assurance requirements. The following must be included as requirements in the special instructions, to be submitted as part of the offerors' proposals:

1. QA Program Plan--The offeror must include as part of the proposal the following information concerning management of the QA Program for the proposed project:

   a. A statement of policy concerning the organization's commitment to implementing a QA program to ensure generation of measurement data of adequate quality to meet contract requirements.

   b. An organizational chart showing the position of the designated QA officer or coordinator within the organization. This QA person should, if possible, be independent of the functional groups that generate measurement data.

   c. A delineation of the authority and responsibilities of the QA person and the data quality responsibilities of the functional groups of the organization.

2. QA Project Plan--The offeror may be required to submit, as part of the technical proposal, a proposed QA Project Plan for the effort required by the contract. The QA Project Plan must include the QA program of proposed subcontractors. If not required as part of the technical proposal, a QA Project Plan must be submitted as a separate, identifiable part of the awardee's work plan. The QA Project Plan must be completed and approved by the QA officer before the awardee initiates full-scale operations.

   EPA's Guidelines and Specifications for Preparing QA Project Plans[5] should be included by reference in the RFP. QA Project Plans must address the following:

   a. Title page, with provision for approval signatures

   b. Table of contents

   c. Project description (experimental design)

   d. Project organization(s) and responsibilities

   e. QA/QC objectives for measurement data, in terms of precision, accuracy, completeness, representativeness, and comparability

   f. Personnel (adequacy of training and experience)

   g. Facilities, services, equipment, and supplies

   h. Recordkeeping

i. Chain-of-custody

j. Sample collection

k. Sample analysis

l. Data processing, analysis, validation, and reporting

m. Specific procedures to be used to routinely assess data precision, representativeness, comparability, accuracy, and completeness of the specific measurement parameters involved (this section is required for all QA Project Plans)

n. Internal QC checks and frequency

o. QA performance and systems audits, and frequency

p. Calibration procedures, references, and frequency

q. Preventive maintenance procedures and schedules

r. Documentation control

s. Configuration control

t. Feedback and corrective action

u. QA reports to management

3. QA Performance Audits--The offeror must agree to participate in performance audits using selected QC reference samples or devices, or to document recent performance on such samples. Such reference samples or devices will be supplied by EPA at no cost to the offerors. The inclusion of requirements for performance audits in the RFP and contract will depend on the availability of QA reference materials or devices for the measurements to be made. Performance audits are required where samples are available, unless the offeror has previously met this requirement to the satisfaction of the QA officer. In the event that routine QC reference materials or devices are not available for the measurements involved, consideration should be given to the use of common or split samples for cross-comparisons of results from offerors with those of EPA. (The requirement for preaward QA performance audits may be limited to offerors in the competitive range.)

4. QA Systems Audit--The offeror must agree to permit a QA systems audit by EPA of the offeror's facilities, organization, and operations as part of the preaward evaluation and during the period of contract performance. (The requirement for preaward QA systems audits may be limited to offerors in the competitive range.)

#### 4.1.3.2 QA Evaluation of the Proposal--

The project officer and the QA officer are responsible for review of the proposal for QA aspects. Each of the preaward requirements listed above must be included in the RFP evaluation criteria. The offeror must ensure that the requirements for QA Project Plans, QA performance audits, and QA systems audits are met by any proposed subcontractors. The proposal should contain a statement of the bidding institution's policy on QC/QA programs. This should include a description of previous performance on similar projects and program effectiveness in those projects. In particular, there should be an explicit response to the QA requirements in the RFP.

Each proposal should be evaluated against the same set of criteria. Section IV of Form QAR-C (Appendix B) is designed to assist in the evaluation of the following QA aspects of the offeror's proposal:

1. QA Program Plan
2. QA Project Plan
3. QA Performance Audit
4. QA Systems Audit

The relative weight of each required QA factor is determined and entered on the form so that the total of the required QA items is 100. In turn, the relative importance of each QA item is specified in relation to the total of all evaluation factors.

#### 4.1.3.3 QA Requirements for Awarded Contracts--

In reviewing the QA requirements for the awarded contract, the project officer must, in accordance with Section 4.1.3.1, specify the QA requirements of the project for the duration of the contract. The contractor is required to ensure that the QA requirements of the contract are met by all subcontractors. A complete and detailed QA Project Plan is required as a part of the contract, or incorporated therein by reference, to be submitted as a deliverable item. The QA Project Plan requires approval by the project officer and the QA officer. Deviations from, or changes in, the approved QA Project Plan during the project term must be documented and submitted to the QA officer for review and approval prior to their implementation.

4.1.3.3.1 QA requirements in the contract--A completed Form QAR-C indicates that all preaward QA requirements have been fulfilled. The project officer must forward the completed form to the contracting officer for inclusion in the contract file.

4.1.3.3.2 QA reports--Provision for adequate QA reporting must be made by the project officer and the QA officer. Contracts of short duration (i.e., one year or less) may require only a final QA report. Contracts of longer duration may require periodic (e.g., quarterly) QA reports. These QA reports should be separate from other contractually required reports and should contain such information as:

1. Status of QA Project Plan
2. Measures of data quality from the project
3. Significant quality problems, quality accomplishments, and status of corrective actions
4. Results of QA performance audits
5. Results of QA systems audits
6. Assessment of data quality in terms of precision, accuracy, completeness, representativeness, and comparability
7. Quality-related training.

4.1.3.3.3 Level of effort contracts--If the awarded contract is a work assignment or technical directive contract, every work assignment (or technical directive) issued under the contract involving environmentally related measurements must have a QA Project Plan approved by the project officer and QA officer before measurements are initiated. As a minimum, the procedures to be used to assess data precision, accuracy, representativeness, completeness, and comparability of the specific measurement parameters must be addressed.

4.1.3.3.4 QA review of contracts--The QA officer and the project officer must review and evaluate the effectiveness of QA Project Plans throughout the project term. Upon completion of the project, the QA officer and project officer must assess the actual performance of the planned activities and subsequent results. The QA officer must review and approve the final QA project report.

### 4.1.4 Interagency Agreements

Although interagency agreements do not involve RFPs, the QA requirements are similar to those for awarded contracts. Therefore, the discussions and considerations included under Section 4.1.3 should be referred to. Form QAR-IA[4] (see Appendix B) should be used by project officers in QA planning and review of interagency agreements.

## 4.2 PROJECT PLANNING

The quality of research data is strongly affected by the "weak link" phenomenon. If experimental design, equipment maintenance, and data analysis are excellent, but quality of the sample analysis is poor, the overall quality of task data is lowered. Similarly, competent technical skills, excellent facilities, or documented procedures cannot compensate for poor experimental design. It is therefore of paramount importance that all aspects of the project that may impact on data quality be considered in project planning. QA Project Plans should address all items in the project officer's QA/QC Checklist (Figure 4-1), as detailed in Appendix A of this section.

A comprehensive QA program should also include Good Laboratory Practice (GLP) concepts where appropriate. The Food and Drug Administration's (FDA) Good Laboratory Practice Regulations[6] apply to nonclinical studies performed on animals, plants, microorganisms, or subparts thereof. The EPA's proposed Good Laboratory Practice Standards[7] (shown in Figure 4-2) are for use in development of data on the health effects of chemical substances and mixtures tested in accordance with Section 4 of the Toxic Substances Control Act. The proposed EPA GLPs generally apply to all animal bioassay laboratory health effects studies conducted by or on behalf of manufacturers of chemical substances.

It should be emphasized that compliance with the data quality requirements outlined in either the FDA or proposed EPA GLPs does not in itself constitute an adequate QA Program or necessarily ensure the production of high quality research data. An effective QA program must also have the active support of both management and task personnel.

During all phases of protocol development, the project officer should consult with HERL-RTP support personnel (e.g., statisticians, animal care

1. The proposed GLPs apply to studies relating to health and safety evaluations conducted under Section 4 of the Toxic Substances Control Act, whether conducted by the sponsor or under contract or grant. Fourteen terms are defined in this section.

2. Test and control substances must be characterized by strength, purity, composition, and stability before initiation of a study. Their containers must be labeled by name, chemical abstract number or code number, batch number (expiration date), and storage conditions requirements. Handling procedures must be used that ensure proper identification and minimize contamination, deterioration, or damage. Mixtures must be suitably analyzed to characterize their uniformity, concentration, and stability: expiration date is that of the earliest expiring component.

3. An ample number of personnel having adequate and documented education, training, and/or experience must be available to the study. Their personal habits, health, and clothing must be appropriate for their assigned duties. The designated study director ensures that all provisions of the GLPs are fulfilled for the study. The QA unit independently assures management that the facilities, equipment, personnel, methods, practices, records, and controls conform with the GLPs, in each phase of the study, at no more than 3-month intervals.

4. Facilities must be of suitable size, construction, and location to facilitate proper conduct of the study. For animal studies, animals must be properly separated, isolated, and quarantined. Separate areas are required for:

    - biohazardous substances;
    - diagnosis, treatment, and control of known or suspected laboratory animal diseases;
    - sanitary disposal;
    - feed, bedding, supplies, and equipment;
    - handling of test and control substances and their mixing;
    - routine procedures;
    - administrative and personnel use; and
    - secure archival of raw data and specimens.

5. Equipment must be suitably designed and located for operation, inspection, cleaning, maintenence, and calibration according to written procedures; written records are kept to document these operations.

6. Testing facility operation must be by written standard operating procedures (SOP) for (as a minimum):

    - animal room preparation;
    - animal care;
    - test and control substance handling;
    - test system observations;
    - lab tests;

(continued)

Figure 4-2. Summary of EPA's proposed GLPs for health effects.[7]

- handling of moribund/dead animals;
- necropsy;
- specimen collection and identification;
- histopathology;
- data handling, storage, and retrieval;
- equipment maintenence and calibration; and
- transfer, placement, and identification of animals.

All deviations must be authorized by the study director and documented in the raw data. Each lab must have immediately available, suitable lab manuals and SOPs, both active and historical. Reagents and solutions must be labeled to indicate identity, concentration, storage requirements, and expiration date. SOPs for animal care include housing, feeding, handling, care, receiving quarantine, health parameters, and identification. In addition, periodic feed and water analysis must be documented as part of the raw data; cages and racks must be cleaned at appropriate intervals. Bedding, cleaning materials, and pest controls must be documented as noninterfering in the study.

7. Minimum protocol specifications are given (as in the HERL QA guidelines document). The conduct of the study is detailed in terms of the protocol, specimen identity, records, and data recording.

8,9. Reserved.

10. Minimum contents of the final report are outlined (as in the HERL QA guidelines document). Archival of all raw data, protocols, specimens, and final reports is detailed. Indexed, orderly, and secure storage is required for at least 10 years.

11. Inspection of the testing facility must be permitted to an employee of EPA or FDA at reasonable times and manner: for records and specimens, not including QA records.

Appendix A

Additional guidelines are given as follows:

Handling of test substances: DHEW's "Guidelines for the Laboratory Use of Toxic Substances Posing a Potential Occupational Carcinogenic Risk" and IARC's "A Manual on the Safety of Handling Carcinogens in the Laboratory."

Handling of radioactive materials: NRC's Title 10 of CFR.

Administrative and personnel facilities: OSHA's Title 29 of CFR.

Animal care and handling: HEW's "Guide for the Care and Use of Laboratory Animals."

Animal care facilities: HEW's "Guide for the Care and Use of Laboratory Animals," and 9 CFR Part 3.

Figure 4-2 (continued)

coordinator, human exposure chamber project officer) who will be involved in the project. The research protocol, when completed, should include statistical design of the experiment, the data collection and analysis procedures, and appropriate QC and QA measures.

### 4.2.1 Research Plan

It is not a QA function to evaluate the merit of the proposed approach to research. However, it is necessary that a well-defined protocol be provided to determine if the QC efforts are properly placed and timed. In any HERL-RTP task that involves data collection and analysis, it is important to consult a statistician during the initial planning phases of the study and through data collection and analysis. An analysis plan, no matter how ingenious, cannot compensate for a poor experimental design.

A written draft of the protocol for the study should be prepared, including:

1. A statement of objectives and hypotheses to be tested;
2. A description of the experiment, covering the variables to be measured, sample sizes, experimental materials, conditions, and instruments; and
3. An outline of the method of data analysis to be used.

#### 4.2.1.1 Objectives and Hypotheses to be Tested--

A clearly written statement of the research objectives allows precise formulation of the specific hypotheses to be tested. The statement should be specific, avoiding vagueness or excessive ambition. It is advisable to prioritize objectives. The reference population to which the results are to apply should be clearly defined.

#### 4.2.1.2 The Experimental Design--

The experimental design should produce a clear definition of all the variables to be considered, the size of the testing program, the experimental subjects (e.g., animals, cell cultures, humans) and exactly what data are to be collected. A well-designed testing program should answer the following questions:

1. Are all the relevant intrinsic factors (e.g., age, size, weight, sex, reproductive condition) and extrinsic environmental factors (e.g., temperature, duration of exposure,

light-dark cycle, chemical form of the pollutant tested, synergistic interactions) being considered?

2. Are the effects of the relevant variables adequately distinguishable from the effects of other variables, or would it be preferable to smooth over the effect of one or more variables by choice of design (e.g., factorial or randomized design)?

3. Has the possibility of interaction between variables been anticipated and accounted for?

4. Is the experimental design as free of bias as possible?

5. Is the experimental design consistent with the stated objectives? Will the project yield enough data (degrees of freedom) to estimate the main effects and interactions of interest, with precision sufficient for effects to be statistically significant? Are sample sizes justified on the basis of precision using historic or conjectured estimates of variances?

6. Is the experimental design cost effective? Would a more limited design provide equivalent information at lower cost?

7. Does the design make adequate provision for controls (negative, positive, and solvent comparison groups)?

8. Is the design logistically sound? (Are adequate time, space, personnel, etc., available to properly perform the checks necessary to ensure the specified data quality?)

#### 4.2.1.3 Data Analysis--

The research protocol should cover in some detail the proposed method of statistical analysis and the assumed underlying mathematical/probabilistic model. The quality assurance aspect of data analysis primarily involves assessment of accuracy of mathematical operations. Data analysis QA is essentially reduced to software QA and should encompass all means of data manipulation and analysis, whether by hand, calculator, or computer.

### 4.2.2 Personnel

All personnel participating in research-related activities under the auspices of HERL-RTP should possess experience and knowledge adequate to perform the technical tasks assigned. Personnel qualifications should be reviewed and evaluated by the project officer and may be reviewed by the QA officer as well. Professional résumés of key task personnel should be available for these evaluations.

Where appropriate, personnel will be expected to participate regularly in certification programs, including external audit programs for performance evaluation and/or accredited training courses in their areas of specialization. All task personnel should keep abreast of current developments in their fields of expertise. Periodic meetings during task implementation may be helpful in information exchange and lead to improved quality control.

Bench-level personnel should also be involved in the feedback and corrective action loop (Section 4.5.7). This involvement should begin early in the task with a briefing on the overall task goals, methods to be employed, and personnel roles in data quality assurance.

Another aspect relating personnel and data quality is the work attitude of task personnel. Appropriate workloads prevent excessive mental and physical fatigue, and useless effort is avoided with optimum facility and equipment configurations. Proper management techniques result in maximum productivity and data quality. The project officer is in the position to recognize and address those aspects of quality control that create a healthy, motivating atmosphere for task personnel.

### 4.2.3 Facilities, Services, Equipment, and Supplies

Evaluation of task-specific facilities, support services, equipment, and supplies is the responsibility of the project officer in cooperation with the HERL-RTP QA organization. The QA officer may, at his/her option or at the request of the project officer, inspect and evaluate or request an audit by qualified personnel of facilities, support services, equipment, and supplies used by laboratories performing HERL-RTP-supported work.

#### 4.2.3.1 Facilities--

All HERL-RTP-supported facilities should be capable of producing acceptable data quality in an efficient, cost-effective manner with minimum risk to personnel.

The suitability of a facility for the execution of both the technical and QA aspects of a task may be assessed prior to use through a systems audit by qualified technical and QA personnel. These audits should determine if facilities are of adequate size, with satisfactory lighting, ventilation, temperature, noise levels, and humidity, and if they are operationally consistent with their designed purpose. Satisfactory personnel safety and health maintenance features should also be present. HERL-RTP requires that all facilities meet acceptable safety and health standards.[8]

Facility security should be tailored to the task research needs and to personnel safety requirements. Security may range from areas available for common use by nontask personnel to restricted areas accessible only to authorized task personnel.

Authorization and documentation of all changes in facility configuration should be limited to a single professional staff member (e.g., project officer) who is qualified to ensure that necessary modifications will not jeopardize data quality or personnel health and safety within the facility.

#### 4.2.3.2 Support Services--

The reliability of required support services is of primary importance in evaluating the task facilities. Numerous measurement processes depend on routine services (i.e., gases, electricity, heat, steam, or water) and loss of these may cause significant deterioration of data production or quality. Adequate provisions for backup support services should therefore exist.

#### 4.2.3.3 Equipment--

All equipment should be evaluated prior to use for its applicability to the HERL-RTP task. Under the HERL-RTP QA Program, the relationships of all measurement methods and the variables to be monitored should be well characterized and documented before being approved for use. Similarly, the design and performance of equipment should be thoroughly evaluated with the aid of a professional who has both a theoretical and a practical understanding of the specific instrument operation. In some cases, such as for atmospheric analyzers, comparative studies of different manufacturers' equipment have been conducted by EPA or its contractors. These data should be taken into consideration in light of precision and accuracy requirements for the task. Definitive statements about the performance of different manufacturers' equipment cannot be based reliably on examinations of single pieces from each manufacturer. Acceptance testing for new equipment should be performed on an item-by-item basis and documented for comparison with future testing. All testing programs should be designed to determine the optimum operating range of the equipment. Equipment performance should be evaluated periodically by systems and performance audits.

To ensure consistently high data quality in the HERL-RTP program, a plan for routine inspection and preventive maintenance (PM) should be developed and followed for all equipment. Scheduling of a particular PM program should be based on the identification of critical components that are most likely to fail and the overall effect of equipment failure on data quality.

All maintenance activities should be performed by suitably qualified technical personnel using accepted, documented procedures according to the PM plan. The desirability of full- or part-time equipment operator and/or maintenance support should be considered. Frequently, sophisticated instrumentation performs poorly or not at all when many occasional users have access to it. On the other hand, minor but frequent maintenance often keeps an instrument operating at peak performance. In such cases, the cost of a full-time dedicated operator is justified.

Documentation of all scheduled and unscheduled maintenance is essential to monitoring and documenting data quality. Permanent records of the maintenance histories of all equipment, including detailed descriptions of all adjustments made, parts replaced, etc., should be kept in individual bound notebooks, dated, and signed by the proper authority.

#### 4.2.3.4 Supplies--

A well-documented acceptance testing program for all incoming expendable supplies should be adhered to. This acceptance screening ensures that supplies not meeting task specifications are not used. The results of a successful acceptance test confirm: (1) that the substance fully corresponds to the manufacturer's specifications; and (2) that known or suspected interferents are absent. Acceptance screening under the HERL-RTP QA Program involves two classes of consumables: chemicals and biological materials.

4.2.3.4.1 Chemicals--The screening of chemicals or reagent commodities involves verification of assay and examination for impurities. Such screening should be performed on a batch basis using accepted, documented analytical methods. For example, it is necessary to characterize all incoming cylinder gases containing pollutants as to the pollutant concentration and the composition of the diluent gas(es). Following successful completion of the acceptance test, an expiration date should be permanently marked on each container; containers should be stored on a first-in first-out basis.

Permanent labels should be attached to the container with the following information:

Date received: ____________
Date tested: ____________
Date expired: ____________

Storage conditions that will protect the integrity of the material and protect personnel from harmful exposures should be observed. In particular, parameters such as temperature, light, and humidity should be considered.

Recertification should be performed routinely to characterize changes in concentration, formation of new species, or loss of original species to prevent them from degrading task data quality. When possible, the integrity of the substance should be checked prior to each use.

A permanent record of all certification procedures, dated and signed by the appropriate authority, should be kept in a bound laboratory notebook that is filed with the project officer and is accessible to the QA officer.

A well-designed central stockroom tracking system will facilitate rapid reference to the identity of other users of a substance. This is useful for informal sharing of information of interest as well as for rapid identification of users if specific problems (e.g., degradation or contamination) are detected with a particular substance.

Since many chemicals tested at HERL-RTP are potentially hazardous to human health, task personnel should be protected from exposure at all times. When chemicals are known to be toxic, mutagenic, carcinogenic, or teratogenic, the project officer should identify where potential personnel health and safety problems may arise during the completion of the task. Task personnel should be advised of the specific hazards and proper handling procedures for all potentially hazardous chemicals.

4.2.3.4.2 Biological materials--The majority of HERL-RTP research tasks involve the use of biological systems to analyze environmental samples for mutagenicity, toxicity, carcinogenicity, and biochemical response. The biological screening of test materials involves *in vitro* microbial and tissue culture assays, *in vivo* animal assays, and human subjects. These systems present special problems, as biological systems inherently possess a high degree of variation. Because of this inherent variation, QA in this area of research is still in a developmental stage.

## In Vitro Microbial and Cell Culture Lines

*In vitro* microbial strains and cell culture lines that have been quite thoroughly characterized are available for research purposes. The American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 20852, provides:

1. Certified animal cell lines (and is a depository for new animal cell lines),
2. Animal viruses and antisera,
3. Chlamydiae,
4. Richettsiae,
5. Certified pathogenic bacteria.

In addition, the following cultures may be obtained from specific research laboratories:

- Ames/*Salmonella* reverse mutation assay
  Dr. Bruce Ames
  Dept. of Biochemistry
  University of California at Berkeley
  Berkeley, CA 94720

- *E. coli*/pol A DNA damage assay
  Dr. Herbert Rozenkrantz
  Dept. of Microbiology
  NY Medical College
  Valhalla, NY 10595

- Mouse lymphoma mammalian cell culture forward mutation assay
  Dr. Donald Clive
  Burroughs Wellcome
  Research Triangle Park, NC 27709

- Chinese Hamster Ovary (CHO) Cells forward mutation assay CHO/HGPRT
  Dr. Abe Hsie
  Oak Ridge National Laboratory
  P.O. Box Y
  Oak Ridge, TN 37830

- CHO/V79-cell transformation and forward mutation assay
  Dr. Elie Huberman
  Oak Ridge National Laboratory
  P.O. Box Y
  Oak Ridge, TN 37830

Health hazard assessment of environmental chemicals evaluates the possible health hazards associated with those chemicals. The approach HERL-RTP has taken is to use *in vitro* and *in vivo* bioassays in a battery of tests, each test measuring a different endpoint.[9] These tests include microbial, tissue culture, plant, sperm, and whole animal testing. Endpoints measured include mutagenicity, toxicity, carcinogenicity, genetic damage in chromosomes (both somatic and genetic), neurological damage, and related health effects. *In vitro* tests are cost- and time-effective and can identify substances that may then be screened further for possible health effects in more time-consuming and costly *in vivo* animal testing.

## In Vivo Animal Testing

Intermediate between microbial cultures and human subjects are animal subjects (primarily mammals). Inbred animal strains have been quite well characterized for generations and correlate closely with certain aspects of human health. For example, $C_3H$/HeJ mice have been selected for their ability to convert polycyclic aromatic hydrocarbons to their active carcinogenic form.[10] A large portion of HERL-RTP testing involves experimentation with animal subjects.

Specific screening procedures for intramural HERL-RTP animal studies should be developed with the assistance of the HERL-RTP Laboratory Animal Staff (LAS). Adherence to accepted animal handling procedures and animal facility accreditation by the American Association for Accreditation of Laboratory Animal Care (AAALAC) are considered minimum requirements for all HERL-RTP animal studies. Animal selection should be based on awareness of the animal strain's genetically determined immunities, as well as the specific dose-response relationship to be investigated. The research protocol should clearly state the basis for selection of a particular species and strain. Acceptance testing, or prescreening and surveillance, should be sufficiently comprehensive to ensure that only suitable animals are used as experimental subjects and controls. Although the added expense of such testing may limit the quantity of animals used, the increase in data quality will generally more than compensate for this loss.

Comprehensive HERL-RTP guidelines for research involving animals are presented in Section 7. The animal care support facility has a QA Project Plan on file with the QA officer.

Human subjects come from diverse and largely unknown backgrounds. Variability among human subjects can be minimized, but not eliminated, by careful pretest screening and questioning to determine medical history, work history, personal habits, and present health status. Project officers supervising tasks involving human subjects must comply with the existing regulations and guidelines on the protection of human subjects.[11]

### 4.2.4 Recordkeeping

The cardinal principle of recordkeeping for scientific research is that all raw data must be retained in a manner that is secure and that expedites validation and access. Complete, permanent, and chronologically organized records of all project activities should be maintained. All information that might be useful in data analysis and interpretation should be recorded. This includes, in addition to raw data, explicit identification of equipment, reagents and other supplies, experimental subjects (e.g., animals), protocol modifications, and quality control activities. The exact organization of the project records should be specified in the protocol and is subject to approval by the project officer.

A cross-referencing system should be used if the data are to be easily accessible following their initial use. Such a system may be of various levels of complexity, depending on the amount of data collected and their potential applications. Rules for nonclinical laboratory reports and records, and their generation, storage, retrieval, and retention on a long-term basis are available.[12] When data are logged by computers, it is important that adequate provision be made for redundant and physically separate long-term storage of such records (Section 4.4.2).

All technical personnel should be provided with a personal notebook in which they chronologically record all data in dark permanent ink, from weights and temperatures to calculations and general observations. Formats for data should be standardized for the project and not left to individual discretion. Efforts should be made to encourage the entry, not only of specific data (e.g., weights, absorbances, volumes, atmospheric or meteorological conditions, status of instruments, etc.), but also of anecdotal data and comments. Erroneous or invalidated data should be indicated in such a way that the entry is flagged, but remains legible. Drawing a single line through the entry, so that the value is still readable, is an acceptable indication; this flag should be initialed. The reason for suspicion of the datum should be recorded in the comments column. Such information may become extremely valuable in subsequent evaluation of a completed experiment or in initial planning of a related one.

It may be advisable to provide station, laboratory, or task data notebooks, in addition to individual notebooks or project data notebooks, to follow the relationship between project components. Such records will generally take the same form and adhere to the same recommendations as personal notebooks. Related notebooks should be cross-referenced.

Instrument logbooks contain all data relating to a particular piece of equipment. This log maintains, in one location, a chronological record of instrument operation, calibration, maintenance, failures, and idiosyncrasies. Such a record is often useful in determining trends, spare parts inventories, etc. A specific format should be used for such data to minimize the possibility of omission of important procedures or data.

The project officer should check that data logging forms for measured parameters have been designed to ensure complete data, high productivity of technical personnel, and ease of reading the raw data. Data coding forms should be designed in consultation with personnel who must record and evaluate the data. In some cases, a data transfer can be avoided by designing forms in consultation with keypunchers or other data entry personnel. Computerized data acquisition systems have many advantages, but require close monitoring and frequent auditing for erroneous or stray electrical signals. Many systems are able to concurrently produce printed output as well as computer-readable output (usually magnetic tape); where possible, it is advisable to employ both.

High quality recordkeeping serves at least two useful functions: (1) it makes possible the detailed reanalysis of a set of data at a future time when the model has changed significantly, thus increasing the cost-effectiveness of the data; and (2) it may be used in support of the experimental conclusions if various aspects of the study are called into question. This latter point goes to the heart of scientific research: objectively, it is often possible to interpret data in more than one way and the raw data must be available for evaluation by qualified professionals; subjectively, when recordkeeping is careless, suspicion is quickly aroused that all other aspects of the research are of similarly poor quality.

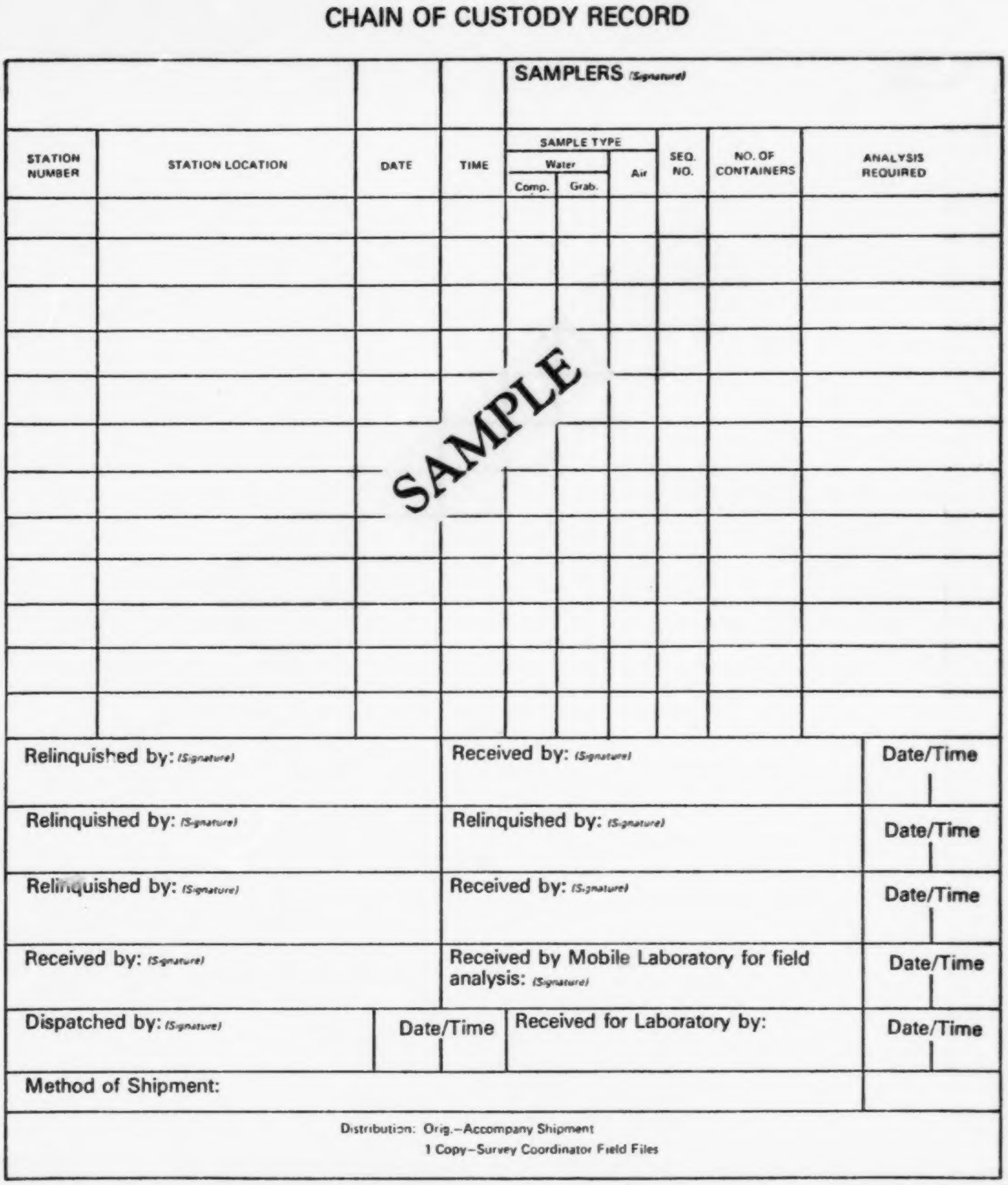
### 4.2.5 Chain-of-Custody Procedures

All projects involving health effects research should document and implement a chain of possession and custody of any sample collected, whether or not the resulting data are to be used in enforcement cases. Such procedures ensure that the samples are collected, transferred, stored, analyzed, and destroyed only by authorized personnel.

#### 4.2.5.1 Field Custody Procedures--

The following chain-of-custody procedures are specifically applicable to large-scale monitoring programs involving shipment of samples from the sampling site to the analysis laboratory.[13] They are intended to be comprehensive and may be used as guidelines for all measurement or research programs. Depending on the specific scope and nature of a project, the project officer should tailor the chain-of-custody procedure to the project. The most important concern is that any chain-of-custody procedure be properly documented and followed for the duration of the task.

1. Samples must be accompanied by a chain-of-custody record that includes the project title, collectors' signatures, collection site, date, time, type of sample, sequence number, number of containers, and analyses required. (An example of a chain-of-custody record is shown in Figure 4-3. Note: Standardized formats should be tailored specifically to each project and used consistently for the duration of the task.) When turning over possession of samples, the transferor and transferee sign, date, and time the record sheet. This record sheet allows transfer of custody of a group of samples from a collection area to the central analysis laboratory. When a custodian transfers a portion of the samples identified on the sheet to the laboratory, the individual samples must be noted in the column with the signature of the person relinquishing the samples. The laboratory person receiving the samples acknowledges receipt by signing in the appropriate column.



**CHAIN OF CUSTODY RECORD**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | DATE | TIME | SAMPLERS (Signature) | | | | | |
| STATION NUMBER | STATION LOCATION | DATE | TIME | SAMPLE TYPE | | | SEQ. NO. | NO. OF CONTAINERS | ANALYSIS REQUIRED |
| | | | | Water | | Air | | | |
| | | | | Comp. | Grab. | | | | |
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SAMPLE

Relinquished by: (Signature) — Received by: (Signature) — Date/Time

Relinquished by: (Signature) — Relinquished by: (Signature) — Date/Time

Relinquished by: (Signature) — Received by: (Signature) — Date/Time

Received by: (Signature) — Received by Mobile Laboratory for field analysis: (Signature) — Date/Time

Dispatched by: (Signature) — Date/Time — Received for Laboratory by: — Date/Time

Method of Shipment:

Distribution: Orig.–Accompany Shipment
1 Copy–Survey Coordinator Field Files

Figure 4-3. Example of a network chain-of-custody record.[12]

2. The collector custodian has the responsibility of packaging and dispatching samples to the laboratory for analysis. The dispatch portion of the chain-of-custody record must be filled out, dated, and signed.

3. To avoid breakage, samples should be carefully packed in shipping containers such as ice chests. The shipping containers are padlocked for shipment to the receiving laboratory. Special shipping precautions are necessary for toxic or hazardous materials and must conform to Federal regulations.[14 17]

4. Packages must be accompanied by the chain-of-custody record showing identification of the contents. The original record must accompany the shipment. A completed copy is retained by the project officer after completion of the analysis.

5. If sent by mail, register the package with return receipt requested. If sent by common carrier, a Government bill of lading should be obtained. Receipts from post offices and bills of lading should be retained as part of the permanent chain-of-custody documentation.

6. If delivered to the laboratory when appropriate personnel are not there to receive them, the samples must be locked in a designated area within the laboratory so that no one can tamper with them, or they must be placed in a secure area. The recipient must return to the laboratory, unlock the samples, and deliver them to the appropriate custodian.

#### 4.2.5.2 Laboratory Custody Procedures--

Suitable laboratory chain-of-custody procedures include:[13]

1. The laboratory should designate a sample custodian and an alternate custodian to act in his/her absence. In addition, the laboratory should set aside a sample storage security area. This should be a clean, dry, isolated room with sufficient refrigerator space that can be securely locked from the outside.

2. Samples should be handled by the minimum possible number of persons.

3. Incoming samples should be received only by the custodian, who will indicate receipt by signing the chain-of-custody record sheet accompanying the samples and retaining the sheet as a permanent record. Couriers picking up samples at the airport or post office shall sign jointly with the laboratory custodian.

4. Immediately upon receipt, the custodian places the samples in the sample room, which will be locked at all times except

when samples are removed or replaced by the custodian. Only the custodian should have access to the sample storage room.

5. The custodian should ensure that all samples are properly stored and maintained under appropriate environmental conditions (i.e., temperature, humidity, light intensity).

6. Only the custodian should distribute samples to task personnel who are to perform analyses.

7. In the laboratory notebook or analytical worksheet, the analyst records information describing the sample, the procedures performed, and the results of the analysis. The notes should be dated, should indicate who performed the tests, and should include any abnormalities that occurred during the testing procedure. The notes should be retained as a permanent record in the laboratory. In the event that the person who performed the tests is not available as a witness at the time of a trial, the Government may be able to introduce the notes in evidence under the Federal Business Records Act.

8. Approved methods of laboratory analyses should be used and documented on all samples.

9. Laboratory personnel are responsible for the care and custody of a sample once it is handed to them and should be prepared to testify that the sample was in their possession and view or secured in the laboratory at all times from the moment it was received from the custodian until the analyses were completed.

10. The laboratory area should be maintained as a secured area and should be restricted to use by authorized personnel only.

11. Once the sample analyses are completed, the unused portion of the sample, together with identifying labels and other documentation, should be returned to the custodian. The returned, tagged sample should be retained in the custody room until permission to destroy the sample is received by the custodian.

12. Samples should be destroyed only upon the order of the project officer in consultation with the QA officer and only if it is certain that the sample is no longer required. The same destruction procedure is true for tags and laboratory records.

#### 4.2.5.3 Evidentiary Considerations--

As accurate and reliable environmentally related measurements become increasingly important in documentation of environmental conditions of public health concern, organizations collecting these data must address evidentiary considerations.

Recording all chain-of-custody procedures and promulgated analytical procedures in writing will facilitate the admission of evidence under Rule 803(6) of the Federal Rules of Evidence (Public Law 93-575). Under this statute, written records of regularly conducted business activities may be introduced into evidence as an exception to the hearsay rule without the testimony of the person(s) who made the record. Although it would be preferable, it is not always possible for the individuals who collected, kept, and analyzed samples to testify in court. In addition, if the opposing party does not intend to contest the integrity of the sample or testing evidence, admission under Rule 803(6) can save a great deal of trial time. For these reasons, it is important that the procedures followed in the collection and analyses of evidentiary samples be standardized and described in an instruction manual, which, if need be, can be offered as evidence of the regularly conducted business activity followed by the laboratory or office in generating any given record.[13]

## 4.3 SAMPLE COLLECTION AND ANALYSIS

### 4.3.1 Sample Collection

Collecting a sample that properly represents the environmental conditions present may be the most technically difficult, hazardous, and time-consuming part of the analytical task. Sampling may range from collecting a representative volume of emission gases from a smoke stack to obtaining tissue samples from rats or biological samples from human subjects. In some special cases, extra data may be required to document the collection procedure. In some research systems, separate control samples must also be obtained.

Requirements for optimal data transmission during sample collection include:

1. Correctly identifying the material or subject to be sampled.
2. Having appropriately trained personnel perform the collection.
3. Having a feedback loop to the project officer or principal investigator regarding problems of sample collection.

Written instructions for sample collection and handling are essential and should include:

1. Project title.
2. Type of sample required.
3. Amount of sample required.
4. Description of sampling procedure.
5. Special processing or handling steps to avoid sample degradation.
6. Test subject preparations or environmental site preparations and precautions.

These instructions should be systematically documented and should be accessible to all personnel.

For the laboratory phase of sample handling, various in-house specimen processing steps (i.e., handling, preservation procedures, precautions, and distribution to analysts) should be documented. In addition, criteria for rejecting inadequate or inappropriate samples should be defined at the initiation of the task.

### 4.3.2 Sample Analysis

Sample analysis often involves a repeated sequence of similar, documented operations by technical personnel and/or automated instrumentation. For this reason, sample analysis is amenable to the use of quality control techniques. The best available operating procedures used by trained technical personnel are the norm in a laboratory research context. Quality control activities require planning by the project officer and may include the analysis of blind samples on a regular basis, samples spiked with known amounts of the analyte to serve as a check on analytical bias, split-sample aliquots analyzed by different analysts at different times using a different set of reagents, and frequent calibration checks using standard samples and blanks.

QC measurements requiring highly developed subjective evaluations (e.g., pathological evaluation of tissue) may require side-by-side or round-robin analysis to establish the quality of the data. The project officer should choose the QC activities appropriate to a given task that will provide highest quality data given the existing analytical limitations.

## 4.4 DATA MANAGEMENT

Following the collection, storage, backup, and validation of raw data, a series of transfers generally occurs prior to data reduction and preparatory to data analysis, interpretation, and reporting. Each of these aspects of the data analysis/processing regime must be addressed in the project plan, together with associated QA measures and their documentation. Validation of computerized data against raw data is discussed in Section 4.5.6; the remaining aspects of data management are discussed below.

### 4.4.1 Data Collection

A clear description of the manner in which raw data are collected is essential to QA planning. These data, representing the actual measured parameters in chronological sequence, subsequently may be flagged as invalid but are never destroyed or deleted. Manually collected data are frequently monitored by the person recording the data. However, computerized data acquisition systems do not have the potential for this treatment and are known to pick up false voltage transients, which may bias the data. In more complex systems, careful analysis of data trends and of the relationships between various parameters may be used to establish windows or intervals within which valid data are expected to occur. It must be recognized, however, that evaluating data to determine if they lie in an expected range does not alone constitute adequate validation. Clearly, data can appear in such an interval, but still involve considerable error.

The use of computerized data acquisition systems is increasing. This frequently permits a statistically acceptable, cost-effective extension of the control chart concept for real-time data validation. There are several advantages to using such a system. Raw data are transformed into tabular or graphical form, thus minimizing human error.[18] Similarly, the capability of rapidly and automatically comparing experimental data against recent values of similar data can serve as a real-time check on data validity.

In all cases, methods for assessing the validity of the recorded raw data as compared with measured or observed data must be established prior to task initiation and documented in the project report (see Section 4.5.6). It is a QA function to evaluate the adequacy of these methods with respect to time, place, and documentation.

### 4.4.2 Data Storage and Backup

Raw data must be stored in such a way that they are not degraded or compromised and that any datum (value) desired may be retrieved (uniquely identified). For computerized raw data, there must always be at least one copy that is off-line and not machine mounted. It is a common practice of large computation centers to provide this service with regularly scheduled backups for users renting on-line disk space. The user should know when such backups are performed. Duplication of user-owned tapes is usually the responsibility of the user.

Raw task data must be securely archived. Such aspects as storage media (e.g., paper, punched cards or paper tape, magnetic tape or disk), conditions, and location must be addressed. Access by authorized personnel and retention time must also be addressed in the QA Project Plan. For certain types of studies, EPA's proposed Good Laboratory Practices[7] are quite explicit.

The storage media, conditions, and locations should be selected based on task-specific criteria. For example, storage location and condition may be inalterably determined for administrative reasons, which would then imply storage media requirements. Another example might be the use and "exercising" of computer files (tape or disk) due to their rather high volatility. Physically separate storage of duplicate raw data sets should be considered. The retention time will vary, but it should be clearly stated at the outset of any study.

Access to the archived data should be described. The fewer persons allowed access, the less chance there is of losing the data. This may conflict with the need to disseminate data to a wide audience. In such a case, copies of data may be provided rather than permitting free access to the unrecoverable raw data.

Another aspect of data storage that should be addressed is data inviolability. Raw data must never be altered. It may be copied, and the copy altered in such a way that an audit trail is generated. An audit trail is an account of the data (values, pages, keypunch forms, keypunch cards, etc.) and a verification after each operation on the data that the number of data items fed into the process is reliably reflected by (usually equal to) the number of data items resulting from the process.

It is not necessary that all data sets created from the raw data set be saved or backed up. In fact, if a second or later generation data set is retained without sufficient documentation to explain how it was created, then that data set can be of little or no value for QA purposes. Thus, more important than retention of intermediate data sets or analyses is the adequate documentation of the procedures used. All computer code (including control language), which accomplishes objectives planned in the protocol, or results to be cited in the final report should be saved.

### 4.4.3 Data Transfers

Data transfer changes the form or location of a data set, but not its content. Thus, a transferred data set may be used to fully reconstruct its originating data set. If data transfer is error free, then no information is lost in the transfer and the input is completely recoverable from the output. Examples of data transfer are copying the raw data from the notebook onto a data form for keypunching, converting a written data set to punched cards, or copying from a computer tape to disk.

A good general rule is to minimize the number of data transfer steps in the data processing, since the overall probability for errors increases with the number of such transformations. Often this can be influenced by judicious design or choice of data forms. For instance, the reliability of keypunchers and other data entry personnel is highly dependent upon the form and legibility of the data they receive. They should be consulted in advance and, insofar as possible, forms should be designed to accommodate them; standardized 80-column Fortran coding forms (6X28-7327-6 IBM) are often desirable. In some cases, it is possible to initially record the raw data on the same form used for data entry (keypunching) or in computer-readable form, which is highly desirable.

As part of the study design, an overall admissible transfer error rate should be specified. The purpose of data validation is to test whether this error rate has been exceeded. If the transfer process has several components, their individual error rates are not of particular concern, as long as the composite error rate is below the desired level. Data validation is discussed in Section 4.5.6.

### 4.4.4 Data Reduction

Data reduction includes all processes that transform one data set to another in such a way that the original data set cannot be recovered from the reduced data set. It is distinct from data transfer in that it entails a reduction in the size (or dimensionality) of the data set and an associated loss of information. Assumptions about the distribution of the observations are implicit in data reduction, making it a data analysis activity. For instance, if repeated measurements of a quantity are made in the laboratory and summarized as a mean and standard deviation, then statistical theory can be invoked to justify the sufficiency of these two measurements, if the data follow a normal distribution.

If the data are reduced before analysis, the study documentation or data management analysis scheme must clearly define the mathematical or other processes used to obtain the reduced data set from the raw data set. Quality assurance should address the accuracy of the mathematical operations used in the reduction process.

Permanent data reduction, resulting in irretrievable loss of raw data, must not occur on EPA projects.

### 4.4.5 Software

The objective of software quality assurance is to ensure that calculator and computer programs perform accurately and as planned. Such operations should introduce no more than negligible error (e.g., 1 percent or less) relative to the intrinsic variation in the measured processes. For manual calculations, an example should be given in which actual raw data are transformed and can be checked by reviewers. If a programmable calculator is used in this process, a copy of the programs used should be provided.

Computer programs should be designed to expedite validation. Programs should be modular, structured, well documented, logical, and should liberally employ comment statements. The use of widely available statistical analysis packages such as SAS, BMD, SPSS, and MINI-TAB is recommended, as opposed to writing analysis programs in FORTRAN, BASIC, or PL/I code. Such packages are heavily used, so that errors have been largely eliminated, and standard documentation is widely available.

The following minimal documentation is sufficient for computerized data manipulation or analysis:

1. Reference to system documentation (some software packages supply this automatically);
2. A copy of the calling program and resulting output;
3. A concise, clearly written description of the operand data set and how it derives from the raw data and the operation or analysis to be performed; these may be embedded in the beginning of the program as a comment statement; and
4. A data dictionary defining the variables as they pertain to the operation or analysis as described in item 3 above; the data dictionary may be embedded in 3.

Compliance with items 1 through 4 has no implications for validity of the analyzed data (see Section 4.5.6) or appropriateness of the statistical methodology employed; they must each be addressed separately (see Section 4.4.6).

### 4.4.6 Data Analysis

Data analysis involves statistical comparison of a model, be it a linear model, a dispersion model, an infectivity model, or a legally defined pollutant dose-response threshold, against observed data. It frequently includes computation of summary statistics and their standard errors, confidence intervals, tests of hypotheses about the parameters, and model validation (goodness-of-fit tests). The QA Project Plan should outline a tentative approach to data analysis, enumerate potential problems in the data analysis schemes, and suggest methods of addressing the problems.

It is unlikely that any real-world phenomenon can be perfectly represented by a simple model. Neglected factors, noise, round-off error, incompetence, and other contaminations can degrade data quality. Often a data set can be perfectly summarized by a function, just as some polynomial can be found to pass through data pairs at different values of the independent variable; the predictive value of such a function is, however, questionable, since it essentially just summarizes the data. Typically, there are so many functions available for fitting a given data set that it is impossible to decide among them on the basis of goodness-of-fit alone. A reasonable reduction of the problem is to first restrict attention to models that are scientifically based, in that the model parameters are identified with essential substantive features of the phenomenon. Even then, the same danger exists with scientifically based models if they contain so many parameters that they allow (near) perfect fit of any data set. Thus, the objective of the modeling process should be to arrive at a scientifically plausible model that has a limited number of parameters.[19]

Some common numeric and statistical problem areas are:

- Treatment of missing and partially missing data (e.g., censored data).
- Selection of a small number of most informative variables from among an unmanageable number of variables (e.g., stepwise regression, principal components).
- Multiple comparisons and resulting inflated significance levels (e.g., two independent hypothesis tests, each at an individual .05 = 1 - .95 significance level, have together a composite .10 = 1 -(.95) (.95) significance level).
- Outliers and other model inaccuracies.[20]
- Numeric problems (e.g., attempting to invert an ill-conditioned matrix involving highly correlated variables, or using iterative procedures to solve systems of equations).

The project plan should specifically address any of these problems relevant to the project.

Data analysis QA consists of determining whether the analyses proposed in the protocol were in fact carried out and, if so, if they were performed correctly. Complete study documentation is required for this. The model-building process for a large-scale study may be so involved that a statistician functioning in a QA capacity cannot check on all planned analyses. In such a case, sampling inspection principles may be applied, leading to review of a subset of the study documentation.

### 4.4.7 Reporting

The most visible product of a research task is the report of important findings. Publication guidelines applicable to the HERL-RTP research reports are available[21] [22] and minimum technical contents for nonclinical laboratory reports and health effects research have been promulgated[7] and are shown in Figure 4-4.

1. Name and address of the facility performing the study and the dates on which the study was initiated and completed.
2. Objectives and procedures stated in the sponsor-approved protocol, including any changes in the original protocol including justification(s).
3. Statistical methods employed for analyzing the data.
4. The test and control substances identified by name, chemical abstract (CAS) number or code number, strength, purity, and composition or other appropriate characteristics.
5. Stability of the test and control substances under the conditions of administration and storage.
6. A description of the methods used.
7. A description of the test system used. Where applicable, the final report must include the number of animals used, sex, body weight range, source of supply, species, strain and substrain, age, and procedures used for identification.
8. A description of the dosage, dosage regimen, route of administration, and duration.
9. A description of all circumstances that may have affected the quality or integrity of the data.
10. The name of the study director, the names of the other scientists or professionals, and the names of all supervisory personnel involved in the study.
11. A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis.
12. The signed and dated reports of each of the individual scientists or other professionals involved in the study.
13. The locations where all specimens, raw data, and the final report are to be stored.
14. The statement prepared and signed by the quality assurance unit.

Figure 4-4. Minimum technical report content for EPA health effects tests.[7]

The report should be concise, complete, and consistent with standard EPA formats.[21] [22] Discussion of the important technical aspects of the research should be adequate to permit qualified professionals to duplicate results. Adequate data should be included to permit at least partial calculation of important results. The conclusions, drawn from the data, and the rationale behind those conclusions should be clearly stated. Graphical and illustrative data correlation with supporting tables should be used whenever possible. Well-defined error estimates should be included with all quantitative values reported.

The presentation of results should delineate the functional relationship linking the data and the tables or graphs and be understandable to nonstatisticians. Since any scientific study falls short of realism, useful conclusions usually require generalizations that tend to lie outside the realm of strict statistical justification. Thus, the reader of the technical report should be informed of the amount of statistical and physical justification supporting each conclusion. The purpose(s) and conclusion(s) of the research should be stated clearly. The estimated errors, as well as the limits of applicability of results, should be stated in such a way as to minimize misinterpretation. Application of the results to alternative theories (models) should be provided, with indication of the rationale used in reaching the stated conclusions rather than the alternative conclusions.

Quality control and quality assurance activities should be detailed to permit the specialist and nonspecialist alike to assess correctly the level of the QA effort invested in the research. Subjective evaluation of the validity and accuracy of the reported results and conclusions should be possible from the data presented.

## 4.5 QUALITY CONTROL

### 4.5.1 Internal Audits

The ability of the total data system to produce data of a specified quality should be regularly evaluated to determine if corrective action (see Section 4.5.7) is needed. Internal audits, conducted by the operating group or organization, are used to obtain data for this evaluation.

The Environmental Protection Agency defines two types of audits that perform these functions.[23] [24] A quantitative measure of the quality of the data produced is usually obtained through a performance audit. A qualitative assessment of the ability of a system to produce data of the specified quality is evaluated by means of a systems audit.

Performance audits should be performed by qualified technical personnel not routinely involved in the specific task measurement process being audited. For example, for the Ames/*Salmonella* assay, an audit could be performed by introducing several mutagens of known response into the assay system. The identity of the test sample or samples would be known only to the auditor. The same principles should also be applied to laboratory measurement instrument systems. Frequently, the performance audit can only be designed to evaluate a part of the total data system, such as sampling, analysis, and/or data reduction. In this case, the audit should be designed to evaluate each subsystem to the fullest extent possible. In either case, the audit values are compared with those generated by the data system(s), and conclusions are drawn as to the quality of the data being generated by the total system.

Tools available for use in performance audits generally fall into one of four categories:

1. Reference materials, for accuracy determinations, are available from several sources,[25] [26] [27] most notably the National Bureau of Standards. These may be included for analysis in various types of measurement systems at relatively low cost with little interference to the normal laboratory routine and with the highest possible degree of confidence.

2. Reference devices may be obtained for which the critical parameters are known to the auditor but not the analyst. These may be more disruptive of laboratory operations and there is no possibility of anonymity of the sample; however; the final result is still a measure of the performance of the total analytical system, including the operator.

3. Cooperative analysis, such as round-robin analysis is useful for estimating the precision of measurement among several different operators and/or laboratories. Accuracy of the measurement can only be assessed if the analyte is a reference material.

4. Side-by-side analysis, or collaborative analysis, may be used if important variables are not controllable in the sample.

Systems audits consist of an evaluation of the various components of a research operation principally through onsite visits by qualified professionals. Professional and technical evaluation, resulting from observation and discussion, is made of the capability of a data system (including instruments, personnel, organization) to produce the specified data quality. Use of checklists or written questionnaires will allow more complete, objective assessment during the systems audit. Questions answered on the basis of such observations include:

- Are written sampling and analysis procedures being used?
- Are written calibration procedures used as frequently as necessary?
- Is a defined preventive maintenance schedule followed?
- Are data reduction, validation, and reporting techniques completely documented and routinely used?

It is important to emphasize that the purpose of an audit is to evaluate constructively measurement process data quality (not personnel) and to identify areas where improvements can be made. If this intent is followed by project officers and made clear from the beginning, personnel will be more likely to cooperate in audit and corrective action cycles.

In either situation, the program and rationale for internal audits should be designed based on individual components of the specific measurement process and clearly planned for and budgeted into the task plans. By using internal audits, the project officer will be able to objectively evaluate data quality as the task progresses.

### 4.5.2 Preventive Maintenance

To ensure long-term data quality in a cost-effective manner, a rational preventive maintenance (PM) program must be followed. This assumes importance roughly in proportion to the amount of instrumental data recorded. Reference 23 contains a discussion of preventive maintenance, especially as related to routine measurements (e.g., air quality monitoring). In particular, preventive maintenance will increase the completeness of data from continuous monitoring systems, which is an important measure of quality in such systems.

In a laboratory research environment, PM has the real but less visible benefit of minimizing and controlling equipment downtime and therefore extending the completeness of the data. Preventive maintenance can be budgeted and scheduled based on historical failure analysis data or recommendations of the manufacturer. Extended laboratory use of specific items can be scheduled with higher reliability than if maintenance occurs only following equipment failure.

The laboratory equipment PM program should include scheduling, performance, and recordkeeping. Scheduling of PM should be developed based on the effect of equipment failure on data quality, any relevant site-specific effects, and equipment reliability. This schedule should be available to the person or group responsible for performing the maintenance as well as the person or group using the equipment so that use of the equipment may be scheduled accordingly.

Preventive maintenance should be performed by qualified technicians, using accepted, documented procedures. The specific service should be based on the considerations noted in the preceding paragraph and should be known to both the user and maintenance groups. A predefined set of data should be obtained before and after the maintenance activities to permit equipment performance evaluation. Calibration (see Section 4.5.3) should be performed following all maintenance activities.

Documentation of all maintenance activities--scheduled or not--is essential to monitoring and documenting data quality. A bound notebook (see Section 4.2.3.3) should be kept with each instrument as a record of its maintenance history. A detailed description of all adjustments made and parts replaced should be recorded. If the notebook is the multicopy type, one of the copies should be kept by the maintenance group for analysis. This analysis may include such considerations as mean time between failure (MTBF) for specific components, MTBF analysis for total systems (individual and laboratory-wide), and development of an onsite spare parts inventory to cost-effectively reduce equipment downtime. Where possible, checklists should be used to ensure and document thorough maintenance activities.

### 4.5.3 Calibration

Calibration is the process of establishing the relationship between the output of a measurement system and that of a known input; it allows different instruments to be correlated with each other and with a specified reference standard.[28] Calibration is an integral part of the measurement process and is a major factor in controlling the accuracy of results. Since the reported accuracy of the measurement method can be no better than the accuracy of the calibration system, calibration is also a limiting factor.

A sound calibration system should include provisions for:

1. Selection of the highest quality calibration standard.
2. Detailed documentation of calibration procedures including specifications for reagents, materials, support equipment, and pertinent environmental conditions.
3. Construction of a calibration curve or a corrective table to determine appropriate correction factors.
4. Maintenance of a record of calibration histories for instruments, support equipment, and standards including identification of instruments and standards, dates of calibration, and calibration results.
5. Determination of calibration frequency needed to ensure quality data collection; identification of acceptance limits in terms of precision and accuracy, and corrective action to be taken when limits are exceeded.

#### 4.5.3.1 Calibration Standards--

Calibration standards should be of the highest quality available and fully characterized. In the United States, the National Bureau of Standards (NBS) holds the position of final authority in the preparation of many reference materials. The NBS Standard Reference Material (SRM) series is generally regarded as producing the best standards of each type available.

Investigators should check on the availability of SRMs applicable to their measurement needs. NBS has been rapidly developing suitable SRMs for environmentally related measurements. The National Bureau of Standards provides information on available standards in the regularly revised Special Publication 260,[25] regular publicity releases, and in a special mailing list for newly issued SRMs. In addition, a new monthly column in American Laboratory entitled "Reference Materials," edited by the Deputy Chief of the Office of Standard Reference Materials, is an excellent source of current information on NBS-SRMs. Considering the rapidity with which SRMs are being developed, and the pressing need to compare data to standards of known high quality, this column should be reviewed regularly by each investigator.

Examples of SRMs currently available from NBS that may be applicable to environmental measurement systems include:

- Hydrocarbon Blends
- Primary Working and Secondary Standard Chemicals
- Microchemical Standards
- Metallo-Organic Compounds
- Isotopic Reference Standards
- Radioactivity Standards
- Industrial Hygiene Standards
- Trace Element Standards
- Clinical Laboratory Standards
- Environmental Standards
- Biological Standards
- Certified Physical Properties Standards.

Selected examples of SRMs are shown in Appendix C.

Use of NBS-SRMs completely fulfills the requirement of high quality and full characterization. However, because SRMs are not mass produced and are individually characterized by lot, they are expensive and often in short supply. Therefore, it is generally desirable to employ secondary standards as the actual calibration standards, maintaining an SRM as a final high quality calibration standard for the secondary working calibration standard.

Whenever a secondary standard is employed in a calibration, it is necessary that traceability to a quality primary standard be established and maintained. EPA regulations now require traceability of calibration standards to NBS-SRMs where possible,[29] [30] and it is likely that this requirement will appear in all future regulations.

Unfortunately, for many common measurement processes routinely used at HERL-RTP, there are no SRMs available. In these cases, the investigator must use the best available calibration standard or devise a standard. Such standards must also meet the requirements of high quality and complete characterization applicable to SRMs. Careful characterization of such standards involves rigorous testing to establish the true value of the reference material within known limits of precision. Such rigorous testing may include repeated analysis of the standard material by more than one analyst or technique, or round-robin interlaboratory analyses, to establish the true value.

### 4.5.3.2 Calibration Procedures--

Written calibration procedures describing each step in the process are required. Calibration procedures may be prepared in-house by qualified personnel, derived from instrument or process manufacturer's instructions, or obtained from sources such as the American Society of Testing and Materials[31] or the National Bureau of Standards.[26] Only the most current and acceptable procedures available for the specific calibration should be used. In addition, only personnel familiar with the measurement process and the calibration procedure should perform the calibration.

An aspect of the calibration operation that is often overlooked is the calibration of reagents, materials, and support equipment. Most calibration procedures use equipment and/or reagents in addition to the standard(s). All such reagents, materials, and support equipment should have been subjected to recent calibration prior to use in the standard calibration procedure.

Even for authoritative standards, such as NBS-SRMs, sample integrity may be questionable if proper storage and handling procedures are not observed. Maintenance of environmental conditions should be appropriate to a specific calibration measurement being conducted. Documented environmental conditions must be maintained during the entire calibration procedure. The use and handling of the calibration standard should be of particular concern as potential problems in calibration may arise with mistreatment of otherwise valid calibration standards.

The following list delineates some of the more common problems associated with the use of some standards:

1. Permeation devices should be used and stored under carefully specified environmental conditions of humidity,[32] and temperature,[33] and should be protected from possible environmental contaminants.[32]

2. Certain gases in pressurized cylinders require special procedures for routine installation to prevent cylinder and regulator contamination with atmospheric oxygen or moisture.
3. Electronic standards frequently require periods of several hours for stabilization of output.
4. Most solid standards require conditioning at a specified humidity prior to weighing.

These examples illustrate that users of standards should be familiar with specified environmental conditions pertinent to handling of each standard. It is imperative that this be recognized if high quality data are to be obtained from the measurement process.

#### 4.5.3.3 Analysis of Calibration Data--

Data from a calibration should be summarized as a function,

$$y = f(x) + \text{error}$$

where x denotes correct value and y denotes measured value. A linear relation is often assumed:

$$y = a\,x + b + \text{error}$$

where a is the slope, and b is the intercept of the line. The inverse, $x = g(y) = \frac{(y-b)}{a}$, serves as a correction function.

Data from a calibration should first be plotted and judged as to their linearity. Ideally, tests for quadratic or higher order effects would be performed. Tests of the hypothesis that the correlation coefficient is at least as large as a prespecified value (R) at a certain significance level (s.l.) may be performed as a partial check on linearity. The value of R and s.l. should be selected by the project officer in consultation with a statistician. If the assumption of linearity is valid, the following statistics should be computed: correlation coefficient, slope, intercept, standard error of estimate (square root of estimated error variance, $\sigma_t$), and standard error of slope and intercept. Raw data from calibrations, as well as these summary statistics, should be recorded in the instrumentation history. If the data exhibit nonlinearity in the working range, then the data should be fit with another more appropriate model, or alternative methods should be considered.

For data interpretation, it is useful to plot the estimated slope, intercept, and standard error of estimate on control charts plotting parameter estimates as functions of time.[28]

#### 4.5.3.4 History of Calibration--

Documentation of each calibration and the full history of all calibrations performed on a measurement system must be recorded. Control charts for slope, intercept, and standard error constitute partial graphical histories of results. The history of calibration for a measurement system should include:

1. Dates of calibration;
2. Identification of the standards used;
3. Support equipment, reagents, and devices used;
4. Personnel performing calibration;
5. Pertinent environmental conditions;
6. Results of calibration (raw data and summary statistics); and
7. Corrective action.

#### 4.5.3.5 Corrective Action--

To characterize the dynamics of the system, each measurement system should undergo an initial intensive audit phase in which it is frequently calibrated. The object is to investigate trends in parameters such as slope ($a_t$), intercept ($b_t$), and the error variance ($\sigma_t$). The time subscripts have been included here to indicate the possibility of changes in the measurement system over time. Two important uses of this information are: (1) to determine if corrective action (adjustment or replacement of equipment) is necessary; and (2) to schedule future maintenance and calibration.

Exceeding control limits calls for corrective action, which should be specified by QA personnel. In some cases, these limits follow from an _a priori_ accuracy requirement. In other cases, they may result from observation of the measurement process over a period of time (e.g., the intensive audit phase). If the measurement process is judged to have performed satisfactorily, then the average parameter estimates (e.g., slope, intercept, standard error of estimate), plus or minus three of their standard errors, may serve as future control limits.

#### 4.5.3.6 Calibration Frequency--

As indicated, it is possible to study the behavior of a measurement system over time and to define the limits of acceptable performance. It is thus possible to estimate the probability of unacceptable performance during a time interval of arbitrary length between calibrations. The time between successive calibrations can then be chosen as the maximum time for which the probability of exceedance is acceptably low, as defined by QA.

Another possible approach is to form a loss function that incorporates cost of calibration and loss due to inaccurate measurement. The first term increases, while the second decreases, as a function of calibration frequency. Assuming that calibration resets the error of measurement to zero, the frequency of calibration may be chosen to minimize the expected overall cost function.[28]

These procedures will not anticipate shocks to the measuring system as opposed to trends. Perceptions of operating personnel are the best source for this information and any suspicion that such a shock has occurred should be followed by recalibration.

### 4.5.4 Documentation Control

A QA program should include a system for updating formal documentation of all operating procedures. One system frequently employed uses a standardized indexing format and provides for convenient replacement of pages that may need to be changed within the technical procedures descriptions.[28]

The indexing format should include, at the top of each page, the following information: section number, revision number, date (note that the date given is the date of revision), and page number as shown below:

| | |
|---|---|
| Section No. | 2.12 |
| Revision No. | 0 |
| Date | September 27, 1977 |
| Page | 1 of 5 |

A digital numbering system identifies sections and subsections within the text. New subsections should begin on a new page. This format groups together the pages within a functional subsection to allow for its expansion. Each time a new page is added or expanded within a section, the number of the preceding page should be included, and a letter added to it. A record of the revisions made to a document must be maintained to derive full benefit of documentation control techniques.

The most important elements of a quality assurance program to which document control is applied include procedures for: sampling, calibration, analysis, data collection and reporting, auditing, sample shipping and storage, computational and data validation (including programmed checks when data processing is computerized), and preventive maintenance.

Full control should be maintained of the distribution of documents. A file should be established within the organization showing such pertinent information as: document number, title, branch originating the document, latest issue date, change number, list of persons to whom document is sent, and signatures of persons acknowledging receipt. Whenever a revision is made, the group responsible should issue the revision, together with a revision notice. Obsolete documents should be removed from all files and points of use, returned to the group responsible, and logged in and destroyed. The group responsible should have sole authority to destroy obsolete documents except for one set of originals and revisions.

Revisions may be promulgated by the issue of entire new documents or of individual replacement pages. In the case of minor revisions, pen-and-ink posting on the original document with the action noted on the revision notice is sufficient. The quality control coordinator should be responsible for distributing documents and/or revisions and for obtaining the required signatures.[28]

### 4.5.5 Configuration Control

An adequate program of equipment/hardware configuration control (e.g., equipment location, environment, component alteration and/or replacement) will readily permit tracking all changes that are made to a data-producing system that may affect data quality. This applies to individual instruments as well as to entire data acquisition systems.

Authorization for configuration changes should be limited to one person, preferably the project officer, to ensure that all changes to the facility are properly documented. This documentation is essential for understanding and explaining shifts in data patterns following such changes. All personnel involved in the task should be notified of changes so that any special procedures required by the changes (e.g., recalibration of analyzers) will be initiated. Finally, configuration control will provide a convenient way of ensuring that all preventive maintenance procedures are performed on schedule.

For monitoring systems, any sampling site changes or monitoring instrument replacements should be recorded in a bound, page-numbered notebook reserved for this purpose. Calibration should follow all such changes.

Configuration control for the laboratory environment is fully as important as for extensive monitoring systems. It includes instrument location in the laboratory as well as modifications that affect measurement data. Temporary and/or permanent equipment configuration changes should be made only when the effect is well characterized and has been demonstrated to improve data quality.

### 4.5.6 Data Validation

Data validation has been defined by EPA as: "the process whereby data are filtered and accepted or rejected based on a set of criteria."[24] This process may include any form of manual or computerized checks, but it clearly involves specified criteria. The QC plan should clearly indicate that raw data are not altered and how subsequent data sets are generated, with a clearly defined audit trail.

Validation criteria may include evaluation of the data with respect to physically determined checks (e.g., a record indicating a negative weight is not reasonable). Similarly, as the sophistication of the model increases, relational checks between measured parameters may also be used.

The data set to be statistically analyzed should be compared with the first recorded form of the data to estimate the error rate. The QA officer should request both the raw data and the analysis data set. QA functions involve definition of error and specification of allowable error rates. In the case of a small data set, quality control may consist of item-by-item verification that the error rate does not exceed the allowable limit. For large data sets, data validation should be considered as a hypothesis-testing problem, with type I and II error probabilities[34] chosen by appropriate QA personnel. A random sample of size sufficient to achieve the desired significance level and power should be drawn. This subsample is then compared item by item with the corresponding raw data to determine if the error rate is acceptable. QA requirements may include documentation in the form of a hard copy of the subsample and access to the raw data. Dodge-Romig tables may be employed to determine sample size, if 90 percent power is acceptable.[35]

### 4.5.7 Feedback and Corrective Action

For each task, a system for detecting, reporting, and correcting problems that may be detrimental to data quality must be established. The feedback and corrective action system chosen should accommodate the need for quick response and thorough communication and documentation of the problem and its solution. If a solution is not immediately apparent, direct contact between the project officer and the involved technician is the best approach to corrective action.

An important aspect in improving the potential for effective feedback and corrective action by task personnel is a quality assurance briefing. The purpose of this briefing is to make the task personnel aware of how their individual contributions to the task affect overall data quality. Such briefings should take place during the initial phases of the task and should be continued at specified intervals throughout the task.

The project officer should hold regular summary briefings with technical personnel to discuss problems encountered in the research task. Monthly progress reports and quarterly summary documents also aid in communication of ideas and therefore improve overall data quality.

These activities provide an excellent opportunity to establish and maintain an active employee-management feedback loop. Since bench-level personnel should be the best observers of routine task operations, they are the most likely to detect disturbances that may affect data quality. With an effective feedback loop in operation, management can quickly become aware of fluctuations that might otherwise go undetected. In general, it is important to impress on task personnel that their daily conduct controls total data quality.

Additional feedback systems should be established. For example, the discovery of an impure substance by one investigator should be communicated at once to all other users of the particular substance. This can be facilitated by the use of central stockroom records.

A description of all problems detected, the solutions devised, and estimates of the effect of the problems on data quality should be made available to appropriate management on a regular basis.

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# APPENDIX A

## GUIDELINES FOR PREPARATION OF HERL-RTP PROTOCOLS

A.1 Research Tasks
A.2 Support Tasks

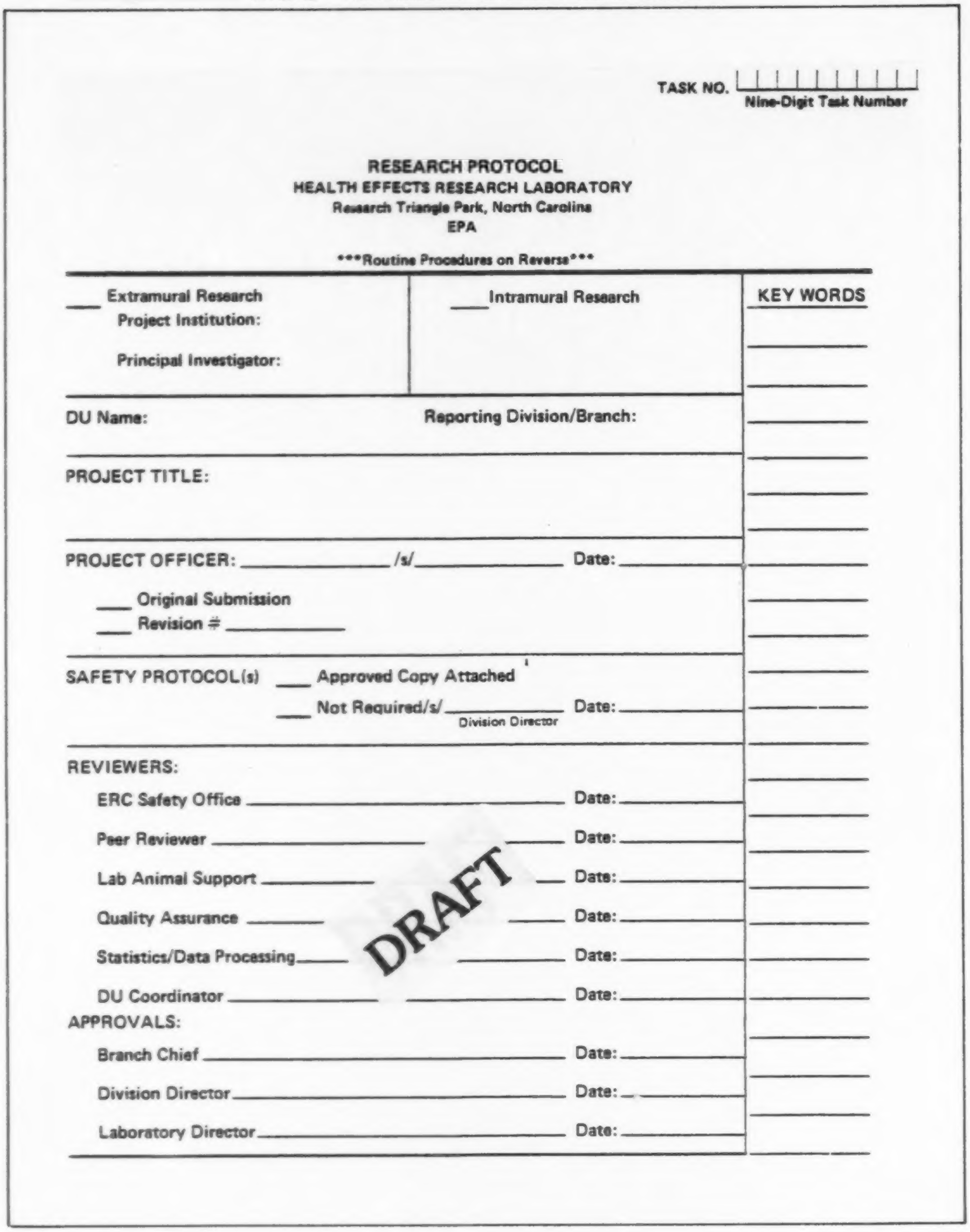
## A.1. GUIDELINES FOR PREPARATION OF PROTOCOLS FOR HERL-RTP RESEARCH TASKS

These guidelines provide a standardized format for use by project officers in preparation of all HERL-RTP research task protocols. Research protocols must include:

1. Cover sheet
2. Summary sheet
3. Technical research plan, including QA Project Plan

Research protocols for all HERL-RTP research tasks must be submitted for review and approval as indicated on the cover sheet. A copy of the approved protocol must be filed with the QA officer.

## A.1.1 RESEARCH PROTOCOL COVERSHEET



TASK NO. ☐☐☐☐☐☐☐☐☐
Nine-Digit Task Number

**RESEARCH PROTOCOL**
**HEALTH EFFECTS RESEARCH LABORATORY**
**Research Triangle Park, North Carolina**
**EPA**

***Routine Procedures on Reverse***

| | | |
|---|---|---|
| ___ Extramural Research<br>Project Institution:<br>Principal Investigator: | ___ Intramural Research | KEY WORDS |

DU Name: Reporting Division/Branch:

PROJECT TITLE:

PROJECT OFFICER: ____________ /s/ ____________ Date: ________

___ Original Submission
___ Revision # ________

SAFETY PROTOCOL(s) ___ Approved Copy Attached
___ Not Required/s/ ____________ Date: ________
Division Director

REVIEWERS:
ERC Safety Office ____________ Date: ________
Peer Reviewer ____________ Date: ________
Lab Animal Support ____________ Date: ________
Quality Assurance ____________ Date: ________
Statistics/Data Processing ____________ Date: ________
DU Coordinator ____________ Date: ________

APPROVALS:
Branch Chief ____________ Date: ________
Division Director ____________ Date: ________
Laboratory Director ____________ Date: ________

DRAFT

- HERL Research Protocol, when required, must include the Health and Safety Protocol.
- The combined protocols will be reviewed as a single entity.

ROUTINE PROCEDURES:

1. Project Officer submits combined protocols to all reviewers.
2. Within 3 weeks, all reviewers will send approved protocols to the Protocol Coordinator (MD-70) who certifies reviewers' signatures and routes through Administrative channels.
3. Protocol Coordinator distributes approved protocols to the Division Directors, Branch Chiefs, Project Officers, and ERC Safety Office.

### A.1.2 SUMMARY SHEET

A one-page summary should be prepared to provide the reviewer with a brief overview of the objectives and approaches of the task. This information should be presented under the following headings.

1. Overall Objective: State clearly and concisely the overall objective of the proposed study.
2. Proposed Means of Meeting Objective: Describe in summary form the means by which the objective will be met. List the components or subtasks that will make up this study.

## A.1.3 TECHNICAL RESEARCH PLAN

1. Introduction: State the overall objectives of the study and summarize briefly the approach to be taken to meet these objectives. Normally a project will be divided into subtasks. List these subtasks and describe each under the following headings: Hypothesis, Proposed Means of Testing the Hypothesis, Experimental Design and Statistical Methodology, and Quality Assurance Project Plan.

2. Hypothesis: State clearly the hypothesis to be tested for the subtask. Include a concise discussion of the facts and/or observations upon which this hypothesis is based, and any alternative hypotheses.

3. Proposed Means of Testing the Hypothesis: Describe clearly the method or methods by which the hypothesis will be tested. Describe each experiment to be performed in sufficient detail to illustrate the relationship of the experiment to the hypothesis. Describe the variables that are to be controlled.

4. Experimental Design and Statistical Methodology: Describe the statistical basis for the collection of data and/or the testing schedule. Determine (estimate) differences in results between test and control measurements that would be accepted as significant; refer to previous work whenever possible to substantiate decisions regarding these differences. Describe measurement design, numbers of measurements, numbers of exposures (i.e., animals to be tested), level of exposure, time of exposure, measurement conditions, etc., that would permit identification of significant differences between test and control measurements in a reasonable period of time and/or in a cost-effective manner.

5. Quality Assurance Project Plan: Describe all QA/QC activities that will be conducted to ensure that the data produced in the task are of adequate and documented quality. The project officer must consider all aspects of the study that may introduce significant variability or are critical to the success of the task, and determine appropriate QA/QC measures and their scheduling. QA Project Plans must address the following:

   a. QA/QC objectives for measurement data, in terms of precision, accuracy, completeness, representativeness, and comparability

   b. Personnel (adequacy of training and experience)

   c. Facilities, services, equipment, and supplies

   d. Recordkeeping

   e. Chain-of-custody

   f. Sample collection

   g. Sample analysis

h. Data processing, analysis, validation, and reporting

i. Specific procedures to be used to routinely assess data precision, representativeness, comparability, accuracy, and completeness of the specific measurement parameters involved. (This section is required for all QA Project Plans.)

j. Internal QC checks and frequency

k. QA performance and systems audits, and frequency

l. Calibration procedures, references, and frequency

m. Preventive maintenance procedures and schedules

n. Documentation control

o. Configuration control

p. Feedback and corrective action

q. QA reports to management

QC activities may also be described in other parts of the protocol and should be clearly identified as such.

6. Support Tasks and Other Activities Required to Successfully Complete This Task: Support tasks and other major resources that will be required to successfully complete the study should be described. These might include exposure measurements, animal care, consultation with regard to statistical treatment of data, and testing the agreement of various models with the data collected. Support tasks should be identified by task number, title, and project officer.

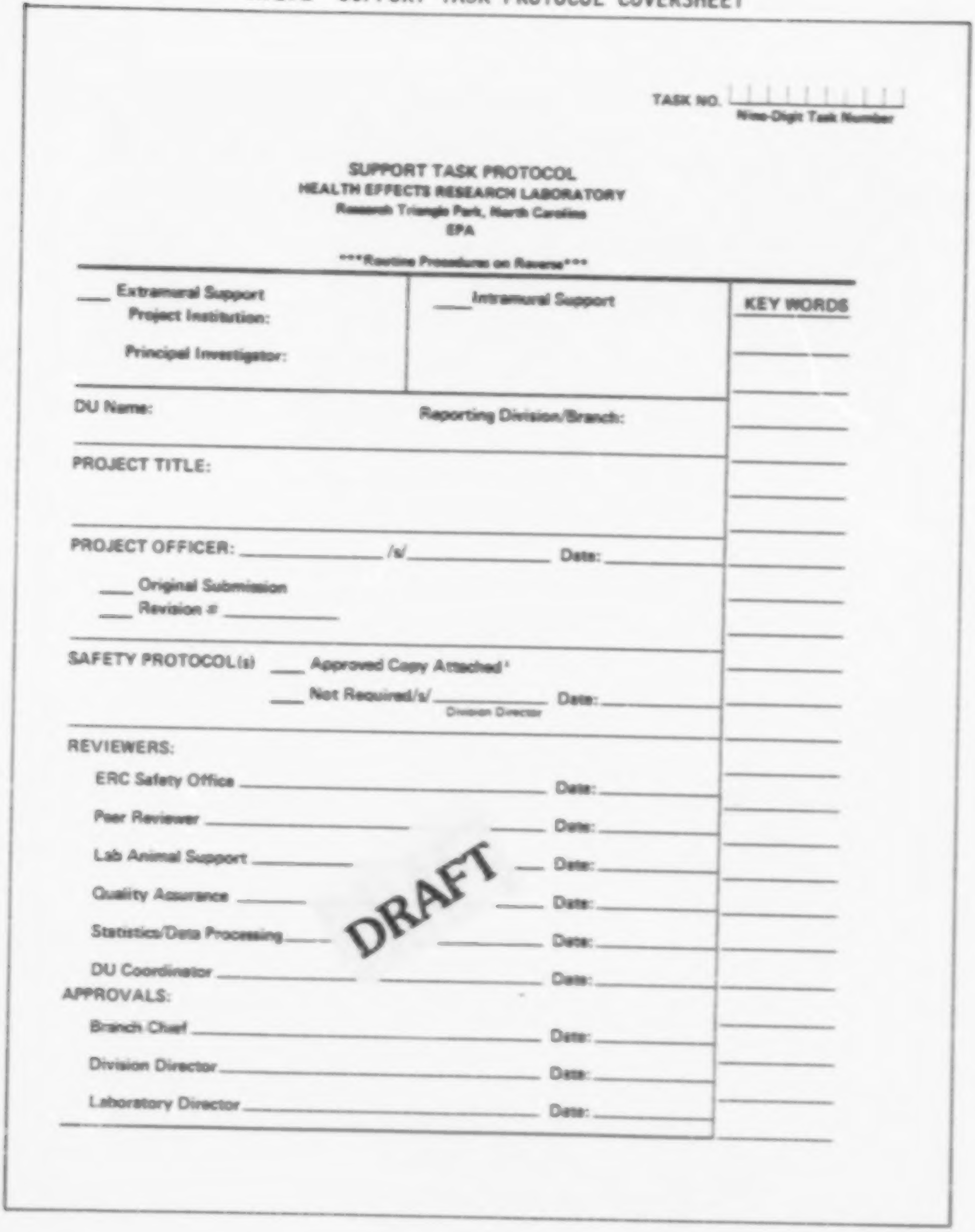
## A.2 GUIDELINES FOR PREPARATION OF PROTOCOLS FOR HERL-RTP SUPPORT TASKS

These guidelines provide a standardized format for use by project officers in preparation of protocols for all HERL-RTP support tasks. Support task protocols must include:

1. Cover sheet
2. Summary sheet
3. Technical project plan, including QA Project Plan.

Protocols must be submitted for review and approval as indicated on the sample protocol cover sheet. A copy of the approved protocol must be filed with the QA officer. Protocols must be revised annually and approved for continued funding.

## A.2.1 SUPPORT TASK PROTOCOL COVERSHEET



TASK NO. ___________
Nine-Digit Task Number

**SUPPORT TASK PROTOCOL**
**HEALTH EFFECTS RESEARCH LABORATORY**
**Research Triangle Park, North Carolina**
**EPA**

***Routine Procedures on Reverse***

___ Extramural Support
Project Institution:
Principal Investigator:

___ Intramural Support

KEY WORDS

DU Name:
Reporting Division/Branch:

PROJECT TITLE:

PROJECT OFFICER: ____________ /s/____________ Date: ________

___ Original Submission
___ Revision # ___________

SAFETY PROTOCOL(s) ___ Approved Copy Attached[1]
___ Not Required/s/ ____________ Date: ________
Division Director

REVIEWERS:

ERC Safety Office ____________________ Date: ________
Peer Reviewer ____________ ______ Date: ________
Lab Animal Support __________ ___ Date: ________
Quality Assurance ________ _____ Date: ________
Statistics/Data Processing ____ ________ Date: ________
DU Coordinator __________ __________ Date: ________

APPROVALS:

Branch Chief ____________________ Date: ________
Division Director ____________________ Date: ________
Laboratory Director ____________________ Date: ________

DRAFT

- HERL Support Task Protocol, when required, must include the Health and Safety Protocol.
- The combined protocols will be reviewed as a single entity.

ROUTINE PROCEDURES:

1. Project Officer submits combined protocols to all reviewers.
2. Within 3 weeks, all reviewers will send approved protocols to the Protocol Coordinator (MD-70) who certifies reviewers' signatures and routes through Administrative channels.
3. Protocol Coordinator distributes approved protocols to the Division Directors, Branch Chiefs, Project Officers, and ERC Safety Office.

### A.2.2 SUMMARY SHEET

A one-page summary should be prepared to provide the reviewer with a brief overview of the objectives and approaches of the support task. This information should be organized as follows:

1. Overall objective: State clearly and concisely the overall objective or purpose of the support task.
2. Proposed means of meeting objective: Briefly summarize the means by which the support task objective will be met. List the specific areas in which support will be maintained and the resources (e.g., facilities, equipment, personnel) available.

## A.2.3 TECHNICAL PROJECT PLAN

1. Introduction: State the overall objective or purpose of the support task and summarize briefly the resources available and approach(es) to be taken to meet the objective. When a support task can be divided into subtasks, list these separately and discuss each under the following headings: Experimental Design and Statistical Methodology, and Quality Assurance Project Plan.

2. Experimental Design and Statistical Methodology: Describe the experimental design or analytical methods to be used to meet the task objectives. Documentation of facilities, equipment, and personnel should include identification and statement of capabilities.

   Where applicable, describe the statistical basis for data collection and/or testing schedules. Specify data quality estimates in terms of accuracy and precision, and determine (estimate) differences in results between test and control measurements that would be accepted as significant. Provide documentation of previous performance whenever possible to support statistical rationale and data quality projections.

3. Quality Assurance Project Plan: Describe all quality assurance/quality control activities that will be conducted to ensure that the data produced are of adequate and documented quality. The project officer must consider all aspects of the support task that may introduce significant variability or are critical to the success of the associated research tasks and determine appropriate QA/QC measures and their scheduling. QA Project Plans must address the following:

   a. QA/QC objectives for measurement data, in terms of precision, accuracy, completeness, representativeness, and comparability

   b. Personnel (adequacy of training and experience)

   c. Facilities, services, equipment, and supplies

   d. Recordkeeping

   e. Chain-of-custody

   f. Sample collection

   g. Sample analysis

   h. Data processing, analysis, validation, and reporting

   i. Specific procedures to be used to routinely assess data precision, representativeness, comparability, accuracy, and completeness of the specific measurement parameters involved. (This section is required for all QA Project Plans.)

   j. Internal QC checks and frequency

k. QA performance and systems audits, and frequency

l. Calibration procedures, references, and frequency

m. Preventive maintenance procedures and schedules

n. Documentation control

o. Configuration control

p. Feedback and corrective action

q. QA reports to management

QC activities may also be described in other parts of the protocol and should be clearly identified as such.

4. Other Activities Required to Successfully Conduct This Task: Other major resources that will be required to successfully conduct the support study should be described.

5. Research Tasks Requiring This Support Task. Identify all research tasks by task number, title, and project officer that are known to be supported by this task.

# APPENDIX B

# QUALITY ASSURANCE REVIEW FORMS FOR EXTRAMURAL PROJECTS

B.1 Contracts (QAR-C, QAMS-002/80)

B.2 Interagency Agreements (QAR-IA, QAMS-002/80)

## B.1 QUALITY ASSURANCE REVIEW FOR EXTRAMURAL PROJECTS (CONTRACTS)

### I. GENERAL INFORMATION

1. Descriptive Title:
2. Sponsoring Program Office: ______________________________
3. Approximate Dollar Amount: ____________________
4. Duration: ______________________________

### II. DESCRIPTION OF WORK

This contract requires the generation of environmental measurements ______ ______
Yes No

### III. QUALITY ASSURANCE (Projects requiring environmental measurements)

| | Yes | No |
|---|---|---|
| 1. Submission of a written QA Program Plan (commitment of the offeror's management to meet the QA requirements of the scope of work) is to be included in the contract proposal. | X | |
| 2. Submission of a written QA Project Plan is to be includ[illegible] the contract proposal. | | |
| 3. A written QA Project Plan is required a[illegible]. | X | |
| 4. Audit samples or devices are av[illegible]nt to this project (see list on rever[illegible] | | |
| 5. Performance on available audit s[illegible]equired as part of the evaluation criteria (see list [illegible] side). If no, attach justification for exception. | | |
| 6. An on-site evaluation of proposer's facilities will be made prior to award to ensure that a QA system is operational and exhibits the capability for successful completion of this project (see schedule on reverse side). | | |

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### IV. DETERMINATION (Projects requiring environmental measurements)

The following will be included in the evaluation of all contract proposals for this project:

| | Yes | No | Relative Weighting |
|---|---|---|---|
| 1. Written QA Program Plan (required) | X | | |
| 2. Written QA Project Plan | | | |
| 3. QA Performance Audit Results | | | |
| 4. QA System Audit Results | | | |
| 5. Other QA Aspects *(Specify)* ____________________ | | | |
| | | | 100 |

Relative weighting of QA to total evaluation ( ):100

QA Form QAR-C

**QA Performance Audits or Split Sample Comparisons**

| | | | | FREQUENCY | |
|---|---|---|---|---|---|
| | Measurement | QC Reference Sample or Device Available | Split Samples for Cross-Comparison | Preaward | During Contract |
| 1. | | | | | |
| 2. | | | | | |
| 3. | | | | | |
| 4. | | | | | |
| 5. | | | | | |
| 6. | | | | | |
| 7. | | | | | |
| 8. | | | | | |
| 9. | | | | | |
| 0. | | | | | |

**Planned Date for QA Systems Audit**

| Preaward | During Contract |
|---|---|
| | |
| | |

**Planned Audit Team**

______________________

______________________

______________________

**QA Reports Schedule**

Progress Reports ______________________

Final Report ______________________

Before submission of the RFP to the Contracts Office, the signatures below verify that the QA requirements have been established.

QA Officer:

Signature Date

Project Officer:

Signature Date

After signatures, a copy of this form should be mailed, with the RFP, to the Contracts Office.

After the selection of the awardee, ______________________, the signatures below verify that the preaward requirements have been satisfactorily fulfilled.

QA Officer:

Signature Date

Project Officer:

Signature Date

After signatures, one copy of the completed form should be mailed to the Quality Assurance Management Staff and one copy to the Contracts Office.

QA Form QAR-C

## B.2 QUALITY ASSURANCE REVIEW FOR EXTRAMURAL PROJECTS (INTERAGENCY AGREEMENTS)

### I. GENERAL INFORMATION

1. Descriptive Title:

2. Sponsoring Program Office: ______________________________

3. Approximate Dollar Amount: ____________________ Total: ______________

4. Duration: ______________________________

### II. DESCRIPTION OF WORK

This agreement requires the generation of environmental measurements ________ Yes ________ No

### III. QUALITY ASSURANCE INTERAGENCY AGREEMENT REQUIREMENTS
(Projects requiring environmental measurements)

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| | | Yes | No |
|---|---|---|---|
| 1. | A written detailed QA Project Plan is requir[illegible]d in the interagency agreement or identifi[illegible] interagency agreement (required) | ________ | ________ |
| 2. | Audit samples or devices are available [illegible]meters relevant to this project (see list on revers[illegible] | ________ | ________ |
| 3. | Performance on available audit samples or devices is required: | | |
| | Prior to agreement | ________ | ________ |
| | During project performance<br>(See schedule on reverse side) | ________ | ________ |
| 4. | An on-site evaluation of performing agency's facilities will be made to determine that a QA system is operational and the capability exists for successful completion of this project: | | |
| | Prior to agreement | ________ | ________ |
| | During project performance<br>(See schedule on reverse side) | ________ | ________ |
| 5. | Periodic and final QA Reports are required | ________ | ________ |

QA Form QAR-IA

**QA Performance Audits or Split Sample Comparisons**

| | Measurement | QC Reference Sample or Device Available | Split Samples for Cross-Comparison | Planned Schedule During Interagency Agreement | |
|---|---|---|---|---|---|
| 1. | | | | | |
| 2. | | | | | |
| 3. | | | | | |
| 4. | | | | | |
| 5. | | | | | |
| 6. | | | | | |
| 7. | | | | | |
| 8. | | | | | |
| 9. | | | | | |
| 0. | | | | | |

**Planned Date for QA Systems Audit**

| Preaward | During Agreements |
|---|---|
| | |
| | |

**Planned Audit Team**

**QA Reports Schedule**

Progress Reports __________

Final Report __________

**QA Officer:**

Signature Date

**Project Officer:**

Signature Date

After signatures, one copy of this completed form should be mailed with the Interagency Agreement request to the Contracts Office, and one copy to the Quality Assurance Management Staff.

QA Form QAR-IA

# APPENDIX C

## SELECTED NATIONAL BUREAU OF STANDARDS STANDARD REFERENCE MATERIALS

Source: National Bureau of Standards, Catalog of NBS Standard Reference Materials for Environmental Research Analysis and Control

### Clinical Laboratory Standards

These SRM's are intended for use in calibrating apparatus and validating analytical methods used in clinical and pathological laboratories, and to assist manufacturers of clinical products in meeting the chemical and physical specifications required for clinical chemicals. (For details on SRM's 930D and 931b, see Spectrophotometric Filters, page 65.)

| SRM | Type | Purity % | Wt/Unit |
|---|---|---|---|
| 900 | Antiepilepsy Drug Level Assay | 4 drugs/3 levels | Set of 4 vials |
| 911a | Cholesterol | 99.8 | 2 g |
| 912 | Urea | 99.7 | 25 g |
| 913 | Uric Acid | 99.7 | 10 g |
| 914 | Creatinine | 99.8 | 10 g |
| 915 | Calcium Carbonate* | 99.9 | 20 g |
| 916 | Bilirubin | 99.0 | 100 mg |
| 917 | D-Glucose | 99.9 | 25 g |
| 918 | Potassium Chloride | 99.9 | 30 g |
| 919 | Sodium Chloride | 99.9 | 30 g |
| 920 | D-Mannitol | 99.8 | 50 g |
| 921 | Cortisol | 98.9 | 1 g |
| 922 | Tris (hydroxymethyl) aminomethane | 99.9 | 25 g |
| 923 | Tris (hydroxymethyl) aminomethane HCl | 99.7 | 35 g |
| 924 | Lithium Carbonate | 100.0 | 30 g |
| 925 | VMA (4-hydroxy-3-methoxymandelic acid) | 99.4 | 1 g |
| 926 | Bovine Serum Albumin (Powder) | ** | 5 g |
| 927 | Bovine Serum Albumin (7% Solution) | ** | 10 vials, 2.15 mL ea. |
| 928 | Lead Nitrate | 100.00 | 30 g |
| 929 | Magnesium Gluconate | IN PREP | 10 g |
| 930D | Glass Filters for Spectrophotometry | + | Set of 3 |
| 931b | Liquid Filters for Spectrophotometry | + | 3 sets of 4 |
| 932 | Quartz Cuvette for Spectrophotometry | + | 1 each |
| 933 | Clinical Laboratory Thermometers | † | Set of 3 |
| 934 | Clinical Laboratory Thermometer | †† | 1 each |
| 935 | Crystalline Potassium Dichromate (UV Absorbance) Standard | (99.972)*** | 15 g |
| 936 | Quinine Sulfate Dihydrate (Fluorescence) | (98.2)*** | 1 g |
| 937 | Iron Metal | 99.90 | 50 g |
| 1968 | Gallium Melting Point | +++ | 1 ea. |

*SRM 915, Calcium Carbonate, was used to develop the first referee method of analysis in clinical chemistry. This work is described in NBS Special Publication 260-36, A Referee Method for the Determination of Calcium in Serum. (See inside of back cover for ordering instructions.)

+Certified for optical properties (see p. 65.)

†Individually calibrated at 0°C and either 25, 30, or 37 °C.

††Individually calibrated at 0, 25, 30, and 37 °C.

**Conforms to NCCLS specification ACC-1.

***Apparent purity, certified for optical properties.

+++Melting Point Certified at 29.7723 °C. (See p. 61.)

## Biological Standards

These SRM's are intended for use in the calibration of apparatus and methods used in the analysis of biological materials for major, minor, and trace elements.

(Values in parentheses are not certified, but are given for information only.)

| SRM | Type | Wt/Unit (grams) | | | |
|---|---|---|---|---|---|
| 1566 | Oyster Tissue IN PREP | — | 1571 | Orchard Leaves | 75 |
| 1567 | Wheat Flour | 80 | 1573 | Tomato Leaves | 70 |
| 1568 | Rice Flour | 80 | 1575 | Pine Needles | 70 |
| 1569 | Brewers Yeast | 50 | 1577 | Bovine Liver | 50 |
| 1570 | Spinach, Trace Elements | 60 | | | |

Content in μg/g (or where noted, wt %)

| ELEMENT \ SRM | 1566 | 1567 | 1568 | 1569 | 1570 | 1571 | 1573 | 1575 | 1577 |
|---|---|---|---|---|---|---|---|---|---|
| Aluminum | — | — | — | — | 870 | — | (0.12%) | 545 | — |
| Antimony | — | — | — | — | (0.04) | 2.9 | — | (0.2) | (0.005) |
| Arsenic | — | (0.006) | 0.41 | — | 0.15 | 10 | 0.27 | 0.21 | 0.055 |
| Barium | — | — | — | — | — | (44) | — | — | — |
| Beryllium | — | — | — | — | — | 0.027 | — | — | (0.017) |
| Bismuth | — | — | — | — | | (0.1) | — | — | — |
| Boron | — | — | — | — | (30) | 33 | (30) | — | — |
| Bromine | — | (9) | (1) | — | (54) | (10) | (26) | (9) | — |
| Cadmium | — | 0.032 | 0.029 | — | (1.5) | 0.11 | (3) | (<0.5) | 0.27 |
| Calcium | — | 0.019% | 0.014% | — | 1.35% | 2.09% | 3.00% | 0.41% | 124 |
| Cerium | — | — | — | — | — | — | (1.6) | (0.4) | — |
| Cesium | — | — | — | — | — | (0.04) | — | — | — |
| Chlorine | — | — | — | — | — | (690) | — | — | (0.27%) |
| Chromium | — | — | — | 2.12 | 4.6 | 2.6 | 4.5 | 2.6 | 0.088 |
| Cobalt | — | — | 0.02 | — | (1.5) | (0.2) | (0.6) | (0.1) | (0.18) |
| Copper | — | 2.0 | 2.2 | — | 12 | 12 | 11 | 3.0 | 193 |
| Europium | — | — | — | — | (0.02) | — | (0.04) | (0.006) | — |
| Fluorine | — | — | — | — | — | (4) | — | — | — |
| Gallium | — | — | — | — | — | (0.08) | — | — | — |
| Indium | — | — | — | — | — | — | — | — | (0.05) |
| Iodine | — | — | — | — | — | (0.17) | — | — | (0.18) |
| Iron | — | 18.3 | 8.7 | — | 550 | 300 | 690 | 200 | 268 |
| Lanthanum | — | — | — | — | (0.37) | — | (0.9) | (0.2) | — |
| Lead | — | — | — | — | 1.2 | 45 | 6.3 | 10.8 | 0.34 |
| Lithium | — | — | — | — | — | (0.6) | — | — | — |
| Magnesium | — | — | — | — | — | 0.62% | (0.7%) | — | 604 |
| Manganese | — | 8.5 | 20.1 | — | 165 | 91 | 238 | 675 | 10.3 |
| Mercury | — | 0.001 | 0.0060 | — | 0.030 | 0.155 | (0.1) | 0.15 | 0.016 |
| Molybdenum | — | (0.4) | (1.6) | — | — | 0.3 | — | — | (3.4) |
| Nickel | — | (0.18) | (0.16) | — | (6) | 1.3 | — | (3.5) | — |
| Nitrogen | — | — | — | — | (5.9%) | 2.76% | (5.0%) | (1.2%) | 10.6% |
| Phosphorus | — | — | — | — | 0.55% | 0.21% | 0.34% | 0.12% | (1.1%) |
| Potassium | — | 0.136% | 0.112% | — | 3.56% | 1.47% | 4.46% | 0.37% | 0.97% |
| Rubidium | — | (1) | (7) | — | 12.1 | 12 | 16.5 | 11.7 | 18.3 |
| Scandium | — | — | — | — | (0.16) | — | (0.13) | (0.03) | — |
| Selenium | — | 1.1 | 0.4 | — | — | 0.08 | — | — | 1.1 |
| Silicon | — | — | — | — | — | — | — | — | (17) |
| Silver | — | — | — | — | — | — | — | — | (0.06) |
| Sodium | — | 8.0 | 6.0 | — | — | 82 | — | — | 0.243% |
| Strontium | — | — | — | — | 87 | 37 | 44.9 | 4.8 | (0.14) |
| Sulfur | — | — | — | — | — | (1900) | — | — | — |
| Tellurium | — | (≥0.002) | (≥0.002) | — | — | (0.01) | — | — | — |
| Thallium | — | — | — | — | (0.03) | — | (0.05) | (0.05) | (0.05) |
| Thorium | — | — | — | — | 0.12 | 0.064 | 0.17 | 0.037 | — |
| Uranium | — | — | — | — | 0.046 | 0.029 | 0.061 | 0.020 | (0.0008) |
| Zinc | — | 10.6 | 19.4 | — | 50 | 25 | 62 | — | 130 |

## Environmental Standards

### Analyzed Gases

These SRM's are intended for the calibration of apparatus used for the measurement of various components in gas mixtures, and in some cases for particular atmospheric pollutants. Each SRM is accurately certified and is primarily intended to monitor and correct for long-term drifts in instruments used. Each cylinder (except 1609) contained 870 liters at STP prior to certification, and thus contains somewhat less than 870 L (SRM 1609 contained 68 liters). All cylinders conform to the appropriate DOT specifications.

| SRM | Type | Nominal Concentrations |
|---|---|---|
| 1609 | Oxygen in Nitrogen | $O_2$, 20.95 mole percent |
| 1658 | Methane in Air | $CH_4$, 0.951 µmol/mol (ppm) |
| 1659 | Methane in Air | $CH_4$, 9.43 µmol/mol (ppm) |
| 1660 | Methane-Propane in Air | $CH_4$, 4.10 µmol/mol (ppm)<br>$C_3H_8$, 0.976 µmol/mol (ppm) |
| 1661 | Sulfur Dioxide in $N_2$ | $SO_2$, 480 µmol/mol (ppm) |
| 1662 | Sulfur Dioxide in $N_2$ | $SO_2$, 942 µmol/mol (ppm) |
| 1663 | Sulfur Dioxide in $N_2$ | $SO_2$, 1497 µmol/mol (ppm) |
| 1664 | Sulfur Dioxide in $N_2$ | $SO_2$, 2521 µmol/mol (ppm) |
| 1665a | Propane in Air | $C_3H_8$, 3 ppm |
| 1666a | Propane in Air | $C_3H_8$, 10 ppm |
| 1667a | Propane in Air | $C_3H_8$, 50 ppm |
| 1668a | Propane in Air | $C_3H_8$, 100 ppm |
| 1669a | Propane in Air | $C_3H_8$, 500 ppm |
| 1673a | Carbon Dioxide in Nitrogen | $CO_2$, 1.0 mol % |
| 1674a | Carbon Dioxide in Nitrogen | $CO_2$, 7.5 mol % |
| 1675a | Carbon Dioxide in Nitrogen | $CO_2$, 15.0 mol % |
| 1677b | Carbon Monoxide in Nitrogen | CO, 10 ppm |
| 1678b | Carbon Monoxide in Nitrogen | CO, 50 ppm |
| 1679b | Carbon Monoxide in Nitrogen | CO, 100 ppm |
| 1680a | Carbon Monoxide in Nitrogen | CO, 500 ppm |
| 1681a | Carbon Monoxide in Nitrogen | CO, 1000 ppm |
| 1683a | Nitric Oxide in Nitrogen | NO, 50 ppm |
| 1684a | Nitric Oxide in Nitrogen | NO, 100 ppm |
| 1685a | Nitric Oxide in Nitrogen | NO, 250 ppm |
| 1686a | Nitric Oxide in Nitrogen | NO, 500 ppm |
| 1687a | Nitric Oxide in Nitrogen | NO, 1000 ppm |
| 2613 | Carbon Monoxide in Air | CO, 18.1 µmol/mol (ppm) |
| 2614 | Carbon Monoxide in Air | CO, 43.0 µmol/mol (ppm) |
| 2619 | Carbon Dioxide in $N_2$ | $CO_2$, 0.5 mol percent |
| 2620 | Carbon Dioxide in $N_2$ | $CO_2$, 1.0 mol percent |
| 2621 | Carbon Dioxide in $N_2$ | $CO_2$, 1.5 mol percent |
| 2622 | Carbon Dioxide in $N_2$ | $CO_2$, 2.0 mol percent |
| 2623 | Carbon Dioxide in $N_2$ | $CO_2$, 2.5 mol percent |
| 2624 | Carbon Dioxide in $N_2$ | $CO_2$, 3.0 mol percent |
| 2625 | Carbon Dioxide in $N_2$ | $CO_2$, 3.5 mol percent |
| 2626 | Carbon Dioxide in $N_2$ | $CO_2$, 4.0 mol percent |

## Analyzed Liquids and Solids

These SRM's are intended for use in the analysis of materials for elements of interest in health or environmental problems. See also: Clinical SRM's page 39, and Industrial Hygiene SRM's page 44.

SINGLE ELEMENT Concentrations:
Weight percent — boldface
Microgram per gram — light face
Nanogram per milliliter — italics

| SRM | Type | Unit Size | Lead | Sulfur | Mercury |
|---|---|---|---|---|---|
| 1579 | Powdered Lead Base Paint | 35 g | **11.87%** | — | — |
| 1620 | Sulfur in Residual Fuel Oil | IN PREP | | | |
| 1621 | Sulfur in Residual Fuel Oil | 100 mL | — | **1.05%** | — |
| 1622a | Sulfur in Residual Fuel Oil | IN PREP | — | — | — |
| 1623a | Sulfur in Residual Fuel Oil | IN PREP | — | — | — |
| 1624 | Sulfur in Distillate Fuel Oil | 100 mL | — | **.211%** | — |
| 1630 | Trace Mercury in Coal | 50 g | — | — | 0.13 μg/g |
| 1641a | Mercury in Water (μg/mL) | IN PREP | — | — | — |
| 1642a | Mercury in Water (ng/mL) | 950 mL | — | — | *1.10* ng/mL |

| SRM | Type | Element Certified | Nominal Concentration | Vol/Unit (mL) |
|---|---|---|---|---|
| 1636 | Lead in Reference Fuel | Pb | 12, 20, 28 and 773 μg/g* | 3 vials each |
| 1637 | Lead in Reference Fuel | Pb | 12, 20, and 28 μg/g* | 4 vials each |
| 1638 | Lead in Reference Fuel | Pb | 773 μg/g* | 12 vials |

*Equivalent grams per gallon are: 0.03, 0.05, 0.07, and 2.0 g/gal, respectively.

MULTI-ELEMENT Concentrations:
Weight percent — boldface
Microgram per gram — light face
Nanogram per gram — italics

| SRM | Type | Unit Size | Al | As | Be |
|---|---|---|---|---|---|
| 1632a | Trace Elements in Coal (Bituminous) | 75 g | **(1.64%)** | 9.3 | — |
| 1633a | Trace Elements in Coal Fly Ash | IN PREP | — | — | — |
| 1634 | Trace Elements in Fuel Oil | 100 mL | — | (0.095) | (<0.01) |
| 1635 | Trace Elements in Coal (Subbituminous) | 75 g | — | .42 | — |
| 1643a | Trace Elements in Water (ng/g) | IN PREP | — | — | — |
| 1645 | River Sediment | 70 g | — | — | — |
| 1646 | Estuarine Sediment | IN PREP | — | — | — |
| 1648 | Urban Particulate | 2 g | — | 115 | — |

| SRM | Cd | Cr | Co | Cu | Fe | Pb | Mn |
|---|---|---|---|---|---|---|---|
| 1632a | 0.17 | 34.4 | (6.8) | 16.5 | 11100 | 12.4 | 28 |
| 1633a | — | — | — | — | — | — | — |
| 1634 | (.01) | (0.09) | — | — | 13.5 | 0.041 | (0.12) |
| 1635 | .03 | 2.5 | (0.65) | 3.6 | 2390 | 1.9 | 21.4 |
| 1643a | — | — | — | — | — | — | — |
| 1645 | 10.2 | **2.96** | — | 109 | **11.3** | 714 | 785 |
| 1646 | — | — | — | — | — | — | — |
| 1648 | 75 | 403 | — | 609 | **3.91** | **0.655** | — |

## Industrial Hygiene Standards

### Organic Solvents on Charcoal

These SRM's consist of charcoal tubes to which have been added known quantities of the specified organic solvent. Each SRM consists of eight tubes, two each of four solvent levels (except 2661a). SRM 2661a consists of nine tubes, three each of three solvent levels. Each tube is color coded for both the solvent and the solvent level.

| SRM | Solvent | Solvent Color Code | Solvent level, mg per tube (Solvent Level Color Code) | | | |
|---|---|---|---|---|---|---|
| | | | I (red) | II (blue) | III (green) | IV (black) |
| 2661a | Benzene | red | 16* | 30* | 54* | |
| 2662 | m-Xylene | blue | 0.040 | 0.293 | 1.79 | 8.38 |
| 2663 | p-Dioxane | green | .016 | .112 | 0.996 | 6.49 |
| 2664 | 1,2-Dichloroethane | white | .098 | .381 | 1.56 | 5.80 |
| 2665 | Chloroform | yellow | .147 | .516 | 2.14 | 6.87 |
| 2666 | Trichloroethylene | black | .286 | 1.03 | 4.09 | 15.4 |
| 2667 | Carbon Tetrachloride | gold | .033 | 0.114 | 0.414 | 1.58 |

*µg per tube

### Freeze-Dried Urine

These SRM's consist of two bottles of freeze-dried human urine, one containing a low and one an elevated level of the element certified.

| SRM | Element | Low Level* (mg/L) | Elevated Level* (mg/L) |
|---|---|---|---|
| 2671 | Fluorine ($F^-$) | 0.835 | 7.14 |
| 2672 | Mercury | 0.0042 | 0.294 |

*When reconstituted with 50 mL water.

### Materials on Filter Media

These SRM's consist of potentially hazardous materials deposited on filters to be used to determine the levels of these materials in industrial atmosphere.

| SRM | Type | Material Certified | Quantity Certified (µg/filter) | | | |
|---|---|---|---|---|---|---|
| | | | I | II | III | IV |
| 2675 | Beryllium on Filter Media | Beryllium | 0.052 | 0.26 | 1.00 | — |
| 2676a | Metals on Filter Media | Cadmium | 1.02 | 2.50 | 10.18 | — |
| | | Lead | 6.96 | 15.23 | 29.64 | — |
| | | Manganese | 1.97 | 9.89 | 19.70 | — |
| | | Zinc | 9.86 | 49.52 | 99.22 | — |
| 2679 | Quartz on Filter Media | Quartz | 3.8 | 29.9 | 76.1 | 193.2 |
| | | Clay | (400) | (370) | (320) | (200) |

## Trace Element Standards

The SRM's listed below were designed for trace chemical analysis, specifically for calibrating instruments and checking analytical techniques and procedures used to determine trace elements in various inorganic matrices. In addition many SRM's certified for chemical composition have one or more constituents certified at or below the 100 $\mu$ g/g level.

**Trace Element Standards (Nominal Concentrations)**

| Element | 607 (ppm) | 610-611 (ppm) | 612-613 (ppm) | 614-615 (ppm) | 616-617 (ppm) |
|---|---|---|---|---|---|
| Antimony | — | — | — | (1.06) | (0.078) |
| Barium | — | — | (41) | — | |
| Boron | — | (351) | (32) | (1.30) | (0.20) |
| Cadmium | — | — | — | (0.55) | — |
| Cerium | — | — | (39) | — | — |
| Chromium | — | (398.5) | (37.8) | (0.99) | — |
| Cobalt | — | (390) | (35.5) | 0.71 | — |
| Copper | — | (444) | (37.7) | 1.34 | (0.65) |
| Dysprosium | — | — | (35) | — | — |
| Erbium | — | — | (39) | — | — |
| Europium | — | — | (36) | (0.99) | — |
| Gadolinium | — | — | (39) | — | — |
| Gallium | — | — | — | (1.3) | (0.23) |
| Gold | — | (25) | (5) | (0.5) | (0.18) |
| Indium | — | — | — | (0.75) | (0.26) |
| Iron | — | 458 | 51 | 13.5 | (11) |
| Lanthanum | — | — | (36) | (0.83) | (0.034) |
| Lead | — | 426 | 38.57 | 2.32 | 1.85 |
| Manganese | — | 485 | (39.6) | (1.41) | 0.62 |
| Molybdenum | — | (422.6) | (36.94) | (0.79) | — |
| Neodymium | — | — | (36) | — | — |
| Nickel | — | 458.7 | 38.8 | (0.95) | — |
| Potassium | — | (461) | (64) | 30 | 29 |
| Rhenium | — | (49.43) | (6.67) | (0.17) | (0.004) |
| Rubidium | 523.90 | 425.7 | 31.4 | 0.855 | 0.0998 |
| Samarium | — | — | (39) | — | — |
| Scandium | — | — | — | (0.59) | (0.026) |
| Silver | — | (254) | 22.0 | 0.42 | — |
| Strontium | 65.485 | 515.5 | 78.4 | 45.8 | 41.72 |
| Thallium | — | (61.8) | 15.7 | 0.269 | (0.0082) |
| Tantalum | — | (447) | (44) | (0.74) | (0.025) |
| Thorium | — | 457.2 | 37.79 | 0.748 | 0.0252 |
| Titanium | — | (437) | (50.1) | (3.1) | (2.5) |
| Uranium | — | 461.5 | 37.38 | 0.823 | 0.0721 |
| Ytterbium | — | — | (42) | — | — |
| Zinc | — | (433) | — | (2.43) | — |

In addition to the 36 elements listed above, the Glass SRM's contain the following 25 elements: As, Be, Bi, Cs, Cl, F, Ge, Hf, Hg, Li, Lu, Mg, Nb, P, Pr, Se, S, Te, Tb, Tm, Sn, W, V, Y, and Zr.

# CERTIFIED PHYSICAL PROPERTIES STANDARDS

## Ion Activity Standards

These SRM's are intended for use in the preparation of solutions for the calibration of specification electrodes. This includes the pH and pD measuring systems.

### pH Standards

These SRM's are furnished as crystals for the preparation of solutions of known hydrogen ion concentration for calibrating and checking the performance of commercially available pH materials and instruments. They are furnished with certificates giving directions for preparation of the solutions and tables of pH values at various temperatures.

SRM's 186Ic and 186IIc, 191 and 192, and 922 and 923, are certified for use in admixture only. At an equimolar (0.025 molal) mixture of SRM's 186Ic and 186IIc, a pH(S) of 6.863 at 25 °C is obtained. Directions also are furnished for the preparation of a physiological reference solution from 186Ic and 186IIc having a pH(S) of 7.415 at 25 °C.

| SRM | Type | pH(S) (at 25 °C) | Wt/Unit (grams) |
|---|---|---|---|
| 185e | Potassium acid phthalate | 4.004 | 60 |
| 186Ic | Potassium dihydrogen phosphate | 6.863 | 30 |
| 186IIc | Disodium hydrogen phosphate | 7.415 | 30 |
| 187b | Borax | 9.183 | 30 |
| 188 | Potassium hydrogen tartrate | 3.557 | 60 |
| 189 | Potassium tetroxalate | 1.679 | 65 |
| 191 | Sodium bicarbonate | 10.01 | 30 |
| 192 | Sodium carbonate | | 30 |
| 922 | Tris(hydroxymethyl)aminomethane | 7.699 | 25 |
| 923 | Tris(hydroxymethyl)aminomethane hydrochloride | | 35 |

### pD Standards

These SRM's are furnished as crystals for preparation of solutions of known deuterium-ion concentration for the calibration and correction of pH indicating equipment to indicate pD data. SRM's 2186I and 2186II, and 2191 and 2192, are certified for use in admixtures only.

| SRM | Type | pD(S) Values | Wt/Unit (grams) |
|---|---|---|---|
| 2186I | Potassium dihydrogen phosphate | 7.43 | 30 |
| 2186II | Disodium hydrogen phosphate | | 30 |
| 2191 | Sodium bicarbonate | 10.74 | 30 |
| 2192 | Sodium carbonate | | 30 |

### Ion-Selective Electrodes

These SRM's are certified for the calibration of ion-selective electrodes and have conventional ionic activities based on the Stokes-Robinson hydration theory for ionic strengths greater than 0.1 mole per liter.

| SRM | Type | Certified Property | Wt/Unit (grams) |
|---|---|---|---|
| 2201 | Sodium Chloride | pNa, pCl | 125 |
| 2202 | Potassium Chloride | pK, pCl | 160 |
| 2203 | Potassium Fluoride | pF | 125 |

# SECTION 5

# EXTERNAL QUALITY ASSURANCE FOR RESEARCH PROJECTS

Every measurement activity supported by HERL-RTP must be performed in accordance with an approved research protocol, including a QA Project Plan and the research hypothesis, on file with the Quality Assurance officer. Objective evaluation of task performance (i.e., application of Good Laboratory Practices (GLPs)) and adherence to QA Project Plans is accomplished through periodic audits by nontask personnel. Such audits may be initiated at the request of the project officer, the QA officer, or the QAMS. They may be conducted on new projects requisite to funding, or on ongoing projects either in response to identified problems in performance or as part of a routine evaluative program. The following discussion focuses on these external quality assurance aspects of the measurement task.

## 5.1 SYSTEMS AUDITS

Systems audits consist of an evaluation of the various components of a research operation, principally through inspection. The first step of a systems audit is an investigation of the laboratory's activities via inspection of protocols, standard operating procedures, proposals, reports, and scientific publications. After inspection of these materials and contact with key laboratory personnel to clarify questions, an onsite inspection may be performed. The onsite systems audit consists of inspection of facilities and operations, interviews with laboratory personnel, and reviews of key operations and documentation. The systems audit may be scored using a checklist comparing actual laboratory practices with some standard such as FDA or proposed EPA GLPs.[1] [2] Scoring schemes may be devised to quantify results of a systems audit.

The objective of the onsite qualitative systems audit is to assess and document: (1) facilities; (2) equipment; (3) personnel; (4) recordkeeping; (5) data validation and management; (6) operation, maintenance, and calibration procedures; and (7) reporting aspects of the total quality control program for a project. The review should:

1. Identify existing system documentation; i.e., maintenance manuals, organizational structure, operating procedures;
2. Evaluate the adequacy of the procedures as documented; and
3. Evaluate the degree of use of and adherence to the documented procedures in day-to-day operations, based on observed conditions and a review of applicable records on file.

From qualitative measures of data quality, an auditor(s) independent of the task organization can assess the suitability of the facilities and operations to meet project goals and identify specific areas where corrective actions may be implemented.

## 5.2 PERFORMANCE AUDITS

Quantitative measurements and comparisons provide the best objective estimates of data quality. The performance audit consists of evaluating the measurement aspects of the laboratory research operation being audited. The laboratory is usually given samples to be analyzed or to be used in some test and their results are compared to expected results and judged for accuracy and precision. A pivotal issue in the proper interpretation of audit results is whether or not high quality reference standards are available.

The National Bureau of Standards has developed a series of environmentally related Standard Reference Materials (NBS-SRMs).[3] [4] A current catalog of NBS-SRMs may be obtained from:

Office of Standard Reference Data
National Bureau of Standards
Washington, DC 20234.

In addition, the World Health Organization maintains information on worldwide sources of biological standards.[5]

Appropriate use of available reference materials by the auditor can provide an objective measure of specific parameter data quality. A variety of techniques, all of which should be designed as blinds (i.e., with operation personnel unaware of the nature of the reference sample), are available. Direct analysis of the reference material and routine duplicate analysis of the samples (one of which is "spiked" with a known amount of the reference material) are two possible uses of reference materials in performance audits of analytical systems.

Unfortunately, NBS-SRMs do not exist for some environmentally related measurements (e.g., known mutagens for audit testing in the Ames/*Salmonella* reverse mutation assay). In such cases, techniques should be devised for probing the quality of the task measurement methods. Round-robin analysis of aliquots of a single sample may be performed by any number of laboratories. Although accuracy (i.e., deviation from a true value) cannot be measured, an estimate of analytical variability (precision) is available. For labile samples, collaborative (side-by-side) analysis may be used. This is equivalent to the round-robin test, but is performed at one location and at approximately the same time. To give a measure of various research system components' variability, interlaboratory and intralaboratory analysis/measurement programs may be designed. In this case, it is important that the statistical design of such testing recognizes such aspects as operating shift changes, diurnal biological changes, and other nonrandom variability in the sample(s) and total measurement system.

After completion of the performance audit, it is useful to present the results to the audited laboratory for review. This review can be a starting point for cooperative identification of sources of measurement weakness and subsequent corrective action.

## 5.3 REFERENCES

1. Department of Health, Education, and Welfare, Food and Drug Administration Nonclinical Laboratory Studies Good Laboratory Practice Regulations, *Federal Register*, *43* (247), December 22, 1978, p. 59986.

2. U.S. Environmental Protection Agency, Proposed Good Laboratory Practice Standards for Health Effects, Title 40, Code of Federal Regulations, Part 772, *Federal Register*, *44* (91), May 9, 1979, p. 27362.

3. National Bureau of Standards, Catalog of NBS Standard Reference Materials, NBS Special Publication 260, U.S. Department of Commerce, Washington, DC.

4. National Bureau of Standards, *NBS Standard Reference Materials for Environmental Research Analysis and Control*, U.S. Department of Commerce.

5. World Health Organization, *Biological Substances: International Standards, Reference Preparations, and Reference Reagents*, Geneva: World Health Organization, 1977.

# SECTION 6

# GUIDELINES FOR ATMOSPHERE GENERATION AND MONITORING

## 6.1 INTRODUCTION

In the HERL-RTP exposure facilities and in population studies, the effects of various atmospheric pollutants on test subjects are evaluated. These pollutants are in gaseous and/or aerosol form. The exposure facilities are used to study the effects of synthetic atmospheres on humans and other test subjects. Population studies evaluate the effects of the ambient atmosphere on humans.

The generation of synthetic atmospheres and the monitoring of both artificial and ambient atmospheres are extremely complex tasks. Guidelines for QA planning for air pollution measurement systems[1] and ambient air methods[2] have been developed by EPA. The goal of these tasks is to produce high quality exposure effects data, hence the details of generation, sampling, and analysis techniques must be considered within the QA Project Plans. In human exposure studies, QA planning begins with subject safety, continues in the experimental tests, and finally provides the basis for estimating the confidence limits on the exposure-effect relationships.

## 6.2 ATMOSPHERE GENERATION

The test atmosphere in an exposure chamber must be well-characterized, in terms of both the composition and the concentration of the components. Exposure experiments may run for a few hours or for several consecutive days. The total dose, as well as the instantaneous concentration level, is important in such experiments. Therefore, it is essential that the exposure source output be stable over the exposure period. Since synergistic effects can complicate interpretation of the experiments, care must be taken to ensure that the desired species are present and that interferents are controlled and/or monitored. Changes in the atmospheric composition due to loss of specific species or generation of another species by physical or chemical reactions must be monitored and documented.

### 6.2.1 General Considerations

Specific test pollutants (gaseous and/or particulate) are produced by a source, mixed with diluent gas, and then introduced into the space surrounding the test subject. Gaseous pollutants are usually obtained from high pressure gas cylinders, although some are produced in situ (e.g., ozone from ultraviolet irradiation). Occasionally, pollutants in ambient concentrations are obtained by dilution of mass emissions from permeation tubes (e.g., $SO_2$, $NO_2$, $H_2S$, $CH_3SH$). Aerosols may be generated by nebulizing a solution or a suspension of known composition. Aerosols in solid form may be obtained from a dust-feeder type of apparatus such as the Wright Dust-Feeder.[3]

To ensure the composition of the atmosphere, the source and the background atmosphere into which the pollutant is released must be stable and well-characterized. Reactivity of the pollutant with the test chamber, including the delivery system, must be characterized and documented.

Since atmospheres are generally prepared by introduction of a specific amount of pollutant into a known volume of background air, knowledge of the quality of this background air is vital. Particulate matter, organic vapors, and other gases should be removed by appropriate filters or adsorbents prior to pollutant introduction. A schedule should be established for the periodic replacement of these filters and adsorbent elements.

One frequently neglected gas is water vapor. The humidity of the test atmosphere is an important variable, especially when atmospheres containing particulate matter are being generated. Surface reactions on particulates and aerosol composition are strongly dependent on the amount of water vapor present. All moisture should be removed from the background air using a mechanical dryer and absorbent. The air can then be rehumidified to a specified level by the addition of steam or a water spray. Test subject humidity requirements must be considered in determining the relative humidity.

Since the test atmosphere is prepared by mixing pollutants and air in proportions determined by the ratios of their volumetric flow rates, the accurate measurement of each of these flow rates becomes critical. Flow measuring devices should be properly calibrated and operated. The pressure and temperature of gases at the flow rate measuring devices must be stable and known. Small absolute errors in the measurement of low pollutant flow rates result in large errors in pollutant concentration. In addition, after pollutant and air flows have been combined, it is important to provide for good mixing of the two components to ensure a homogeneous atmosphere.

Finally, it is important to characterize the test atmosphere as it encounters the test subject, i.e., spatial as well as temporal characterization. This characterization provides data concerning the actual exposure conditions. It is also helpful to characterize the atmosphere as it leaves the source since this information is useful in the early detection of harmful levels of pollutants resulting from source malfunctions. Early detection allows diversion of the defective atmosphere before it reaches the test subject. Interaction between individual components of test atmospheres, always a potential source of error in atmosphere generation, can be minimized by careful attention to parameters such as composition, concentration, and residence time. Interaction between the atmosphere and conduit or chamber walls can also be a source of error. This is especially true for aerosols and reactive gases such as ozone and sulfur dioxide. Even the test subject may interact with the atmosphere in an unexpected and undesirable manner (e.g., $NH_3$ from animal excreta). For valid data to be accumulated from an experiment, each of the interactions that may occur must be carefully examined and controlled by the investigator.

### 6.2.2 Particulate or Aerosol Atmospheres

Atmospheres containing generated particulate matter or aerosols exhibit so many specialized problems that they warrant separate discussion. Since the dose of inhaled particles is mass- and size-dependent, knowledge of both the mass of the particles and their size distribution is necessary to characterize the dose level. If the aerosol atmosphere is a mixture of several particulate components, the size distribution of each should be characterized.

Aerosols may be obtained by nebulization of a solution or a suspension of known composition. Deviations in the aerosol characteristics may result from inadequate flow control, excess loss of solvent, and cooling of the solution due to solvent evaporation. Circulation of the solution from an external large reservoir may be used to avoid the problems due to solvent evaporation. In the Wright Dust-Feeder, lack of homogeneity in the powder plug may produce deviations in aerosol output.[3]

Characteristics of an aerosol may change due to particle-gas or particle-particle interactions. The particle-gas interactions in hygroscopic aerosols result in evaporation or growth of particles.[4] In salt aerosols, humidities above 75 percent will generally result in growth of the particles. This growth process is extremely rapid and can lead to a several-fold particle size increase at high humidities (>90 percent). In acid aerosols, humidities below 20 percent can produce a decrease in particle diameters due to evaporation. As a general rule, and dependent on test subject health parameters, humidity should be maintained constant between 20 and 70 percent to avoid particle growth or evaporation losses.

Particle-particle interactions resulting in coagulation are dependent upon particle size and concentration. Coagulation can lead to significant errors for dense aerosols. In general, if the concentration is less than $10^5$ particles/$cm^3$, coagulation may be neglected. Other factors leading to coagulation are turbulent mixing and extreme polydispersity. Charges on particles also significantly influence aerosol behavior. An aerosol generated by nebulization may require charge neutralization. This will avoid the uncertainty of the effect of charge on particle-particle interaction and deposition on surfaces.

Methods for characterization of aerosol size and concentration are based on a variety of principles. Interconversion between two methods is not usually possible without introduction of significant errors. If an aerosol is used for an inhalation study, the aerodynamic size distribution based on mass is appropriate. To obtain this information, inertial classification of particles by a method such as impaction is necessary. However, in various size ranges, other methods based on electrical mobility, microscopic, or light scattering analysis may be needed to characterize the aerosol. Conversion of data from these methods into aerodynamic size should follow recognized procedures.[5] An estimate of the errors involved should also accompany the conversion.

After the aerosol has been generated and characterized at the source, it is delivered to the test subject. Certain precautions and pretests should be taken to prevent significant change in the atmosphere before it reaches the subject.

Loss of the aerosol component en route to the exposure chamber can be significant. The most common cause of particle loss is deposition on conduit walls. This surface deposition of particles is due to sedimentation, inertia, and diffusion processes: the extent and nature of particle loss is size-dependent. In polydisperse aerosols, deposition loss will affect the particle size distribution of the delivered aerosol as well as the concentration. In general, large particles over a few microns aerodynamic diameter are preferentially lost by sedimentation and inertia. These effects can be minimized by using high flow velocities and by avoiding bends or sudden transitions in conduits (e.g., changes in tubing diameter). Because of this tendency toward deposition, it is extremely important that aerosol atmospheres be finally characterized immediately before they encounter the test subject.

If the atmosphere contains particles larger than 1 μm in diameter, lack of homogeneity in the chamber may be significant. Segregation may occur due to sedimentation or bypassing the inlet and outlet. Distribution of the incoming test atmosphere over as broad an area as possible would minimize the flow channeling problems. Sedimentation effects may be minimized by a vertically downward movement of the test atmosphere. Even with these precautions, segregation may occur. Again, this tendency necessitates characterization of the aerosol at the test subject. The sampling position for this characterization must represent the same location and elevation in the chamber as the test subject. This will assure the characterization of that portion of the atmosphere that actually reaches the subject.

## 6.3 SAMPLE COLLECTION AND ANALYSIS

### 6.3.1 Introduction

Collection of a representative sample is of utmost importance in any measurement process as noted in Section 4.3.1. The analytical results may be of excellent quality; however, if the sample is contaminated, degraded, or is otherwise not representative of the area or population under study, the relationship between the measured pollutant concentration and the response of exposed subjects will not be valid.

It is important to recognize that obtaining a representative sample is difficult, especially when low concentrations of components of the ambient air are measured. For this reason the procedures for sample collection and analysis should be included in the experimental design (see Section 4.2). Sampling methodology and the number of samples required should be established prior to beginning the task.

Ambient air studies frequently deal with large populations and extended airshed areas, which cannot be thoroughly monitored. Thus, statistical sampling techniques are generally required. The number and size of "blanks," control groups, and samples taken from the background should be evaluated carefully. Calibration, instrument spanning, and audits also have an impact on sample collection and analysis efforts.

In most studies, more than one pollutant or parameter will be measured. During the experimental design phase, the requirement for measurement of co-occurring pollutants should be addressed. Important parameters such as humidity, temperature, and atmospheric pressure are also commonly measured.

### 6.3.2 Sample Representativity

A representative sample of ambient air to which plants and animals may be exposed is difficult to obtain. Spatial and temporal aspects of sampling should be considered carefully prior to locating the sampling stations. A thorough background study in support of an ambient air monitoring program should include a study of source inventories, historical meteorology of the area, local topography, and examination of data from any preexisting air monitoring stations.

The point in space from which a sample is taken is an important variable. The sample should be collected in a location that is clearly representative of the air space being characterized. For example, if the objective of the study is to assess the effects of air quality on children, the sampling point might be located 1 to 1.5 meters above the ground in a schoolyard or playground. The inlet to the sampling probe must be located so that it is protected from possible damage by the elements or by vandals, yet is out of the microenvironment of the sampling equipment. The use of mobile sampling equipment is often helpful in locating proper sampling points and in surveying a large area at minimal expense.

The time frame in which a sample is taken also has a bearing on sample representativity. Generally, the longer the period of sampling, the better the sample will characterize the environment. However, the final decisions concerning sampling duration and frequency must be made with respect to the objectives of the task (see Section 4.2). If continuous or semicontinuous analyzers are used, concentration trends and any unusually high or low values will appear when the overall data are examined. On the other hand, if grab samples are collected for short periods of time, it is possible that very high or very low concentrations will be obtained that do not represent the subject's average exposure. If the experiment is well controlled (such as a captured air mass in a chamber), periodic grab sampling can be used. However, even here with certain pollutants the use of grab samples is discouraged due to potential chemical degradation of the sample during transport and storage.[6]

Sample integration is helpful when collecting an air sample for later analysis. In this process the sampling vessel is slowly filled with an air sample over a period of time. Again, sample integration must consider the stability of the sample with time, as well as the averaging of concentration fluctuations.

### 6.3.3 Physical Characterization of the Atmosphere

To obtain an accurate intercomparison of samples taken in various cities or air regimes, it is necessary to know some of the physical characteristics of the ambient or enclosed air mass. Such characteristics include temperature, barometric pressure, relative humidity, and perhaps wind speed, wind direction, and solar radiation. Additionally, knowledge of the temperature, pressure, and humidity within the analytical laboratory is necessary for correction of gas flow rates to standard temperature (25° C) and pressure (1 atmosphere). This is particularly important during the calibration and operation of analyzers and impinger systems.

### 6.3.4 Sample Quantity

A sufficient volume of air must be collected or passed to an instrument to obtain valid data. In the case of continuous analyzers, an excess volume of sample generally flows through a glass sampling manifold and the instrument's sampling line is attached to this manifold. An initial flow rate at least 50 percent in excess of that required by the analyzer(s) is recommended. If the sample flow is less than that demanded by an analyzer, the analyzer or sampling device will pull in room air and the sample will be diluted. Sufficient sample quantity is also needed during calibration. The rate of sample flow to a continuous analyzer should be identical to the flow rate established during calibration. That is, if an analyzer samples 200 $cm^3$/min during calibration, it should sample 200 cm$^3$/min during analysis of ambient air. The same is true of impinger samples in which air is bubbled through a chemical solution. In the case of impingers containing chemical solutions, a sufficient volume of sample must bubble through the solution to achieve a reliably detectable spectrophotometric or other response. For particulate collection devices (high volume samplers, dichotomous samplers, cascade impactors, etc.) the flow rate must be that specified to achieve the entrainment of the desired particle sizes. The particle sampler must sample long enough to build up sufficient deposit for accurate weighing and/or chemical analysis.

### 6.3.5 Sample Handling and Storage

Since many of the pollutants in ambient air are highly reactive, unstable species, they cannot be reliably collected and stored for later analysis. Ozone, oxides of nitrogen, peroxyacetyl nitrate (PAN), sulfur dioxide, and other sulfur species should be delivered directly from the ambient air to the analyzer or impinger through Teflon or glass tubing.

Other less reactive pollutants, such as carbon monoxide and hydrocarbons, may be stored for periods of several days prior to analysis. Teflon or Tedlar bags are adequate for carbon monoxide samples. Stainless steel or glass sampling containers are better for hydrocarbons. There may be no clear consensus in the scientific community as to the reactivity of a specific pollutant. In such cases, it is essential that it be determined and documented as part of the study if the conclusions are to be valid and defensible.

Particulate samples collected on glass fiber or other types of filters are often weighed and analyzed at a later date. For reproducible weight determinations, the filters must be conditioned at a constant relative humidity for a specified period prior to weighing for tare and gross weights. Care must be taken to avoid sample loss during filter handling.

In all cases, stored samples should be protected from unusually high temperatures and intense light. Some samples are preferably stored under refrigeration in the dark.

### 6.3.6 Recommendations for Sampling and Analysis of Selected Pollutants

Recommendations for the sampling and analysis of selected pollutants commonly found in ambient atmospheres follow. Included are suggestions and a summary (Table 6-1) for the six EPA criteria pollutants ($SO_2$, $NO_2$, $O_3$, CO, TSP and Pb) as well as other species of current interest to HERL-RTP personnel. This is not an exhaustive list of pollutants or of sampling and analysis methods. Rather, it is a list of some pollutants of current interest, the most acceptable analysis methods to be used, major interferences, and calibration concepts for additional pollutants.[7]

6.3.6.1 Sulfur Dioxide ($SO_2$)--

The EPA reference method for determination of ambient levels of sulfur dioxide is the pararosaniline method. This manual, wet chemical method is a complex sampling and analysis procedure. EPA has accepted an automated version of this method that reduces the complexity of the analysis.[8] In addition, other continuous instrumental methods are currently available that are officially considered equivalent to the EPA reference method. These methods include coulometric, flame photometric, pulsed fluorescent, and second derivative spectroscopic detection of sulfur dioxide. The coulometric, pulsed fluorescent, and second derivative spectroscopic methods are specific for $SO_2$. The flame photometric method detects sulfur-containing species (e.g., $SO_2$, $H_2S$, R-SH); it can be made specific for $SO_2$ by inserting a scrubber cartridge into the sample inlet line. A technical assistance document on the use of the flame photometric method in ambient air measurements has been published by EPA.[9]

Some nonsulfur compounds do interfere with these methods. It is reported that differences between carbon dioxide concentration in the calibration/zero matrix and the sample matrix interfere with certain flame photometric detectors.[10] Hence, the $CO_2$ concentration in the calibration gas for this instrument should be matched to the $CO_2$ concentration expected in the sample. The pulsed fluorescence method will respond to certain aromatic hydrocarbons unless a special scrubber (e.g., a hydrocarbon cutter) is placed in the sample inlet line.

Calibration of these instruments is usually accomplished against an $SO_2$ permeation device. Gas cylinders of $SO_2$ in $N_2$ or air (widely used for calibrations of source level monitors) may also be used for calibration and span checks of ambient air instruments if the cylinder concentration is approximately 50 ppm or higher, if the concentration is checked versus a higher standard

## TABLE 6-1. SUMMARY OF MEASUREMENT METHODS FOR SELECTED POLLUTANTS

| Pollutant | Measurement method | Interferents | Calibration | Generation |
|---|---|---|---|---|
| Ammonia ($NH_3$) | Indophenol method | Certain particulates | Standard solution of $NH_4^+$ or permeation devices | Cylinder and dilution system |
| | Nitrite method | Particulates containing ammonium salts | | |
| Carbon monoxide (CO)[a] | NDIR (40 CFR, App. C) | $H_2O$, $CO_2$ in high conc. and other IR absorbers, near 2165 $cm^{-1}$ | CO in $N_2$ (cylinders) | Cylinder and dilution system |
| | Methanation/GC-FID | Nonmethane hydrocarbons, depending on scrubber efficiency | | |
| Hydrocarbons (THC) | GC-FID (40 CFR, App. E) | Nonlinear FID response to increasing carbon number | Methane cylinders<br>Propane cylinders | |
| | GC-MS | None | Standard cylinders of pure gases, and mixtures | |
| Hydrogen sulfide ($H_2S$) | Methylene blue ALSO FPD | Strong oxidizing or reducing agents (e.g., $SO_2$) | Permeation system | Cylinder gas dilution with clean air |
| Lead (Pb)[a] | Flame atomic absorption | Light scattering | Standards prepared daily | Dilution of working standard |
| Nitrogen dioxide ($NO_2$)[a] | Chemiluminescence of NO with $O_3$ (40 CFR, App. F) | $CO_2$, $H_2O$[b] PAN[c] | Cylinder NO in $N_2$ and gas phase titration to $NO_2$[d] | Oxidation of NO (from cylinder) by $O_3$<br>$NO_2$ permeation tube |
| Ozone ($O_3$)[a] | UV photometry | High humidity, at low $O_3$ concentrations | UV photometry or acceptable transfer standard | $O_3$ (UV) generator |
| | Chemiluminescence<br>$O_3$-ethylene<br>$O_3$-rhodamine-B | High humidity at low $O_3$ concentrations | UV photometry or acceptable transfer standard | $O_3$ (UV) generator |
| Peroxyacetyl nitrate (PAN) | GC-EC | Varying sample/standard moisture content | Photoanalysis of ethyl nitrite in oxygen[e] | Dilution of generated standard |
| Total suspended particulates (TSP)[a] | High volume sampler (40 CFR, App. B) | Secondary particle formation | High volume sample flow rate is calibrated with ReF device or orifice plates | |
| | | Gas-filter reactions | | |

See footnotes at end of table.

(continued)

## TABLE 6-1 (continued)

| Pollutant | Measurement method | Interferents | Calibration | Generation |
|---|---|---|---|---|
| Sulfates ($SO_4^=$) | Barium methylthymol blue | Anions complexing barium | Standard solution of $SO_4^=$ | |
| | Ion chromatography | | Standard solution of $SO_4^=$ | |
| | FPD with $SO_2$ denuder | | | |
| Sulfur dioxide ($SO_2$)[a] | Pararosaniline (40 CFR, App. A) | Effects of the principal known interferences have been minimized or eliminated as follows: (1) oxides of nitrogen eliminated by sulfamic acid, (2) ozone eliminated by time delay, (3) heavy metals eliminated by EDTA & phosphoric acid | $SO_2$ permeation system | Mixing of high-concentration $SO_2$ with clean air. |
| | Pulsed fluorescence | Aromatic hydrocarbons ("cutter" prevents interference) | $SO_2$ permeation system | Mixing of high-concentration $SO_2$ with clean air. |
| | Flame photometric | Any sulfur-containing species ($H_2S$ scrubber beneficial in removing this interferent) | $SO_2$ permeation system | Mixing of high-concentration $SO_2$ with clean air. |
| | Coulometric | Oxidizing (e.g., $O_3$, $NO_2$) species | $SO_2$ permeation system | Mixing of high-concentration $SO_2$ with clean air. |
| | | Reducing (e.g., $H_2S$) species | $SO_2$ permeation system | Mixing of high-concentration $SO_2$ with clean air. |
| | | Olefins | $SO_2$ permeation system | Mixing of high-concentration $SO_2$ with clean air. |
| Sulfuric acid ($H_2SO_4$) mist | Ion chromatography | $SO_2$ | | |

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[a] EPA criteria pollutants.

[b] Mercer, T. T., Aerosol Technology in Hazard Evaluation, Academic Press, New York, 1973.

[c] Cooper, C., G. Langer, and J. Rosinski, Air Pollution Control Assn. J., 18:57, 1979.

[d] Ellis, E.C., Technical Assistance Document for the Chemiluminescence Measurement of Nitrogen Dioxide, EPA Environmental Monitoring Series, EPA-600/4-75-003, Environmental Protection Agency, Research Triangle Park, NC, December 1975.

[e] Federal Register, December 14, 1977, p. 62971.

every 6 months, and if the tank's contents are carefully diluted with clean, $SO_2$-free air.

### 6.3.6.2 Nitrogen Dioxide ($NO_2$)--

The EPA measurement method for the determination of ambient nitrogen dioxide ($NO_2$) is based on the chemiluminescence produced by the oxidation of NO by ozone. The method is instrumental and continuous. These analyzers detect NO directly and total oxides of nitrogen (NO, $NO_2$, and other N-containing compounds that can be reduced to NO) after passage through a thermal converter. A readout of $NO_2$ concentration is provided indirectly by electronic subtraction. One automated and two manual wet chemical methods have recently been accepted by EPA as equivalent to the reference method.[11] Neither the Christie (arsenite) method nor the TGS-ANSA method is affected by the interferences listed above, but they suffer from the difficulties inherent in all manual sampling and analysis methods.

Recently published research has indicated that the chemiluminescent method is subject to interference from third-body quenching reactions including those with carbon dioxide and water vapor.[12] Research has also shown that the thermal converter (used in this method to reduce $NO_2$ to NO) can reduce nitrogen-containing compounds to NO. PAN is also converted with relatively high efficiency.[13] The efficiency of this converter should be determined frequently, especially when high concentrations of nitrogen dioxide are being analyzed.

Calibration of the NO and $NO_x$ channels of the instrumental method is generally accomplished using bottled standards of nitric oxide in nitrogen. The $NO_2$ channel is calibrated by oxidizing some of the NO calibration standard to $NO_2$ before the gas is introduced into the instrument. This oxidation is accomplished by ozone gas phase titration (GPT). Calibration of this channel may also be accomplished by use of an $NO_2$ permeation device. Much helpful information on the calibration and use of chemiluminescence NO-$NO_2$-$NO_x$ analyzers is available in an EPA technical assistance document.[14]

### 6.3.6.3 Ozone ($O_3$)--

Ozone ($O_3$) is the most commonly measured photochemical oxidant. Wet chemical methods can be employed for measurement of ozone. However, the EPA reference and equivalent methods are the instrumental methods based on ultraviolet photometry, chemiluminescence from the reaction between ozone and ethylene, or chemiluminescence from the reaction between ozone and rhodamine-B.

Calibration is accomplished using a stable ozone source in conjunction with an ultraviolet photometer or a certified transfer standard such as an ozone generator. The response or output of the transfer standard should be determined by ultraviolet photometry. Technical assistance documents are available that discuss the calibration of various ambient air ozone analyzers employing the principle of UV photometry[15] and the methods for establishing certified ozone transfer standards for use in calibration and auditing of monitors.[16]

### 6.3.6.4 Carbon Monoxide (CO)--

The EPA measurement method for continuous monitoring of carbon monoxide in the atmosphere is nondispersive infrared spectrometry (NDIR). The principle is based on the absorption of infrared radiation by carbon monoxide in a nondispersive spectrophotometer. Another method is based on catalytic conversion of carbon monoxide to methane by hydrogenation. The methane is then sensed by a flame ionization detector.

The infrared absorption spectrum of water is sufficiently similar to that of CO to interfere with NDIR measurements. In source level concentrations (i.e., 2000 ppm), $CO_2$ is also an interferent.

Calibration of such analyzers is by injection of carbon monoxide from standard cylinders. Steel cylinders have a tendency to react slowly with carbon monoxide, forming iron carbonyl. Because of this tendency, standards should be verified every 4 to 6 months by comparison to an NBS-traceable standard.

### 6.3.6.5 Total Suspended Particulates (TSP)--

The EPA reference method for total suspended particulate (TSP) is the high volume sampler method. Air is drawn into a covered housing and through a filter by means of a high-flow-rate blower (1.0 to 1.7 $m^3$/min). This flow rate allows suspended particulates having aerodynamic diameters of less than 100 μm to pass to the filter surface. Accurate control of the flow rate is critical to obtaining a valid sample. The collection period for ambient air is generally 24 hours. The filter is conditioned to a fixed relative humidity and weighed before and after sampling. The net weight and total volume sampled are used to estimate average suspended particulate matter in terms of micrograms per cubic meter.

For experiments where smaller volumes of air are available for sampling, low flow rate filters and impactors may be useful. Because the emphasis here will probably be on chemical analysis and not weight, care must be exercised in selecting the filter media or impaction surface to avoid artifact formation and background interference. The possibility of interferences in the analysis should be examined through background and blank analyses. Optical particle counters are available for continuously monitoring the number of particles and, in certain models, the size of particles. Manufacturers of such counters and size discriminators should be able to show how calibration was achieved.

#### 6.3.6.6 Lead (Pb)--

The EPA reference method[17] for the determination of ambient levels of lead is atomic absorption spectrometry using an air-acetylene flame. Lead in particulate matter is solubilized by extraction with nitric acid facilitated by heat or by a mixture of nitric and hydrochloric acids facilitated by ultrasonication.

No major chemical interferences have been reported. If the analyst suspects an interference due to the sample matrix, the interference can be verified and corrected for by using the method of standard additions.

A second type of interference, light scattering, may be observed when using the 217.0 nm lead absorption line. This may be corrected for instrumentally, as described in the Federal Register Method, or by use of the ammonium pyrrolidinecarbodithioate-methylisobutyl ketone (APCD-MIBK) chelation-solvent extraction technique[18] of sample preparation.

Calibration is accomplished using standards prepared daily by dilution of the working standard with the same acid matrix. Standards, in the same acid concentration as the samples, should be selected to cover the linear absorption range indicated by the instrument manufacturer.

#### 6.3.6.7 Hydrocarbons (THC)--

The EPA measurement method for determination of hydrocarbons corrected for methane is based on gas chromatography with flame ionization detection. The method is designed to measure both total hydrocarbons (THC) and methane so that methane can be subtracted from the hydrocarbon analysis. No reference instruments are currently designated because of problems resulting from an inefficient methanator and a nonlinear detector. The instrument is usually calibrated on the basis of methane or propane supplied from low-level standard reference tanks.

If analysis for specific hydrocarbons is sought, the chromatographic column-flame ionization detector approach is preferred. The specific compound is distinguished from others by introducing a known concentration of this hydrocarbon and determining its column retention time. The signal strength from the detector is correlated with concentration by introducing varying known concentrations of the hydrocarbon of interest. Permeation tubes containing certain hydrocarbons may be used to generate standards. Mixtures of hydrocarbons in air or other gases may also be purchased in cylinders.

The possibility of peaks from one or more compounds overlapping during chromatographic analysis increases with the complexity and similarity of the molecules. The extent of this problem should be investigated using several column packings over a range of operating conditions.

Hydrocarbon samples may be collected in Teflon or Tedlar bags for later analysis. However, glass or stainless steel containers are preferred. Certain higher molecular weight hydrocarbons and other organic compounds may be adsorbed on columns of polymeric material such as TENAX-GC and volatilized onto a chromatographic column at a later time.

For maximum reliability in identification of hydrocarbons (and other organic species), the method of choice is the combination of gas chromatography and mass spectrometry.

#### 6.3.6.8 Peroxyacetyl Nitrate (PAN)--

PAN is a photochemical oxidant often found in smoggy atmospheres. Measurement is generally by a gas chromatographic procedure employing an electron capture detector.[19] This method may be subject to interference from low sample moisture content unless the relative humidities of the samples and standards are controlled.[20] [21]

Standards may be synthesized by the photolysis of ethyl nitrite in oxygen.[22] The synthesized standard, however, is not a primary one and must be verified (e.g., by infrared spectroscopy).

### 6.3.6.9 Sulfuric Acid Mist ($H_2SO_4$)--

Sulfuric acid mist may be collected on glass fiber or Teflon membrane filters if it is at low concentration levels (<50 mg/m$^3$) and no sulfur dioxide is present. The sample can then be extracted with deionized water and analyzed by ion chromatography or other acceptable methods. When sulfur dioxide is present, it may be oxidized to sulfate in the presence of water vapor or by oxidants on the filter surface, thus interfering with the acid mist analysis.

### 6.3.6.10 Sulfates ($SO_4^=$)--

Airborne sulfates may be measured by analysis of the particulate matter taken from high volume on dichotomous filters. The technique is generally as described for the measurement of total suspended particulate matter (Section 6.3.6.7). Analysis of the collected particulate for sulfate is then performed using one of several available analytical techniques (e.g., turbidometry, ion chromatography). The analysis method usually recommended is the automated, wet chemical method based on the detection of the barium-methyl-thymol blue chelate.[23] To avoid unwanted formation of sulfates on the filter by reaction of $SO_2$, the pH of the filter must be controlled during manufacture to around pH 5.

### 6.3.6.11 Benzene ($C_6H_6$)--

A tentative method for the determination of benzene in the atmosphere by 24-hour integrated sampling has been established and used by EPA.[24] In this method, a known volume of ambient air is drawn through a tube (NIOSH) containing charcoal at 200 cm$^3$/min for 24 hours; benzene vapors are adsorbed on the charcoal. The charcoal is subsequently transferred to a stoppered container where trapped vapors are desorbed by carbon disulfide. An aliquot of the extract is injected into a gas chromatograph and benzene is quantified by comparing the peak retention time and area to standard solutions of benzene in carbon disulfide. The method's sampling efficiency has been determined to be in excess of 90 percent over a concentration range of 2 to 100 ppb.

### 6.3.6.12 Vinyl Chloride ($C_2H_3Cl$)--

A tentative method for the determination of vinyl chloride in the atmosphere (24-hour integrated sampling) has been established by EPA. In the method, vinyl chloride is adsorbed from ambient air onto charcoal packed in glass tubes. The sample is drawn through the tube at a flow rate of 200 cm$^3$/min for 24 hours. The charcoal is extracted with carbon disulfide. An aliquot of the extract is injected onto a 2.5-m length of 0.4% carbowax 1500/carbopak A column. Vinyl chloride is identified and quantified by comparing peak retention times and peak heights to those determined for standards using the flame ionization detector.

## 6.4 POLLUTANT STANDARDS AND TRACEABILITY

Special emphasis should be placed on the need to characterize all incoming cylinder gases containing pollutants in specified concentrations. The characterization should also include identification of cylinder contents with reference to both pollutants(s) and matrix. It is well known that problems concerning the identity of cylinder contents and accuracy of the specified concentrations are commonplace. Even the best known and most reliable gas suppliers occasionally supply faulty materials. In addition, after the cylinder contents have been initially verified, experience indicates that over a period of time the contents degrade. Therefore, regular recertification must be performed to characterize changes in concentration, formation of new species, or loss of original species to prevent them from degrading task data quality. Because of these considerations, all HERL-RTP gas cylinders should be subjected to a rigorous program of initial, and regularly recurring, certification of contents and concentrations. The reader is referred to the traceability protocol for establishing true concentrations of gases used for calibrations and audits contained in Volume II of the Quality Assurance Handbook for Air Pollution Measurement Systems.[2]

The Environmental Monitoring Systems Laboratory, Quality Assurance Division, maintains a laboratory in Research Triangle Park, NC, for use in validation of cylinder and permeation tube concentration outputs, as well as verification of flow rate apparatus and pollutant generating/dilution systems. For further information on pollutant verification or standards, contact Mr. Thomas Clark, EMSL/QAD, EPA, Research Triangle Park, NC 27709.

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# SECTION 7

# QUALITY CONTROL/QUALITY ASSURANCE GUIDELINES FOR RESEARCH INVOLVING ANIMALS

## 7.1 INTRODUCTION

Research involving animals presents a source of significant data variability--the complex biochemical system of the animal itself. This variability is affected by the animal's genetic, physiological, psychological, and environmental condition. The purpose of this section is to outline procedures to control these variables and thereby assist the investigator in attaining high quality results that are reproducible, accurate, and therefore scientifically valid.

Quality control (QC) begins with experimental design and extends through completion of the final report. The purpose of these QC guidelines is to highlight areas in the research protocol where variance can become significant; it is *not* the purpose of these QC guidelines to dictate or to inhibit the investigator's experimental design. These guidelines address proper QC procedures for the animal staff (Section 7.2) and the research laboratory (Section 7.3). General guidelines for administration of test agents are also included (Section 7.3.2). More specific and detailed procedures are available in the references (see Section 7.5).

These guidelines (Sections 7.2 and 7.3) represent health care standards that are to be met or exceeded by all intramural and extramural HERL-supported animal research activities. They are consistent with the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) Good Laboratory Practice (GLP) regulations.[1] [2] Additional sources were also used,[3-12] with extensive reference being made to HERL-RTP's Laboratory Animal Staff's standard operating procedures, which are revised annually, and contain information on animal support facilities available to the Health Effects Research Laboratory (HERL). Copies can be obtained from HERL-RTP's, Animal Care Coordinator (MD-70).[13] Figure 7-1 is an example of a quality assurance systems audit

## ANIMAL FACILITY CHECKLIST

### Laboratory Animal Management

( ) Caging conditions ______________________
( ) Environmental control ______________________
( ) Caging size ______________________
( ) Sanitary maintenance ______________________
( ) Building maintenance ______________________
( ) Storage of feed ______________________
( ) Identification of animals ______________________
( ) Overcrowding of animals ______________________
( ) Illumination ______________________
( ) Feeding and watering of animals ______________________
( ) Vermin control ______________________
( ) General storage conditions ______________________
( ) Waste disposal ______________________
( ) Physical plant conditions ______________________
( ) Husbandry practices ______________________
( ) Hazardous materials handling and facilities ______________________
( ) Recordkeeping ______________________

### Laboratory Animal Quality and Health

( ) Quarantine and isolation of animals ______________________
( ) Adequate veterinary care ______________________
( ) Diagnosis, treatment and control of animal diseases ______________________
( ) Separation by species ______________________

### Personnel

( ) Personnel health program ______________________
( ) Adequacy of personnel (training and numbers) ______________________
( ) Protective clothing ______________________

### Use of Laboratory Animals

( ) Surgical and postsurgical care ______________________
( ) Euthanasia procedures ______________________

(S) = Satisfactory
(U) = Unsatisfactory
(NA) = Not applicable

Inspection by ______________________

Inspection date ______________ Location ______________________

Figure 7-1. Sample quality assurance inspection checklist for an animal care facility (from AAALAC certification checklist). Section 7.2 should be consulted for details.

checklist for an animal care facility, Figure 7-2 is a QC checklist for animal care procedures and Figure 7-3 is a principal investigator's QC checklist for animal testing.

## 7.2 ANIMAL RESEARCH PROGRAM QUALITY CONTROL

### 7.2.1 Animal Facility Design Quality Control

Quality control guidelines for animal care facilities include but are not limited to:

1. Service areas should include: Animal housing rooms of identical design (dimensions should vary only by suites); an office; a surgery room with adjacent preoperative, scrub, and postoperative areas; a cage wash area and clean cage storage area; feed and bedding storage rooms (separate); a diet kitchen; a diagnostic laboratory; shower and locker rooms; a necropsy room; strategically located receiving, quarantine, and isolation areas; a biohazard suite; a supply storage room; a lounge or lunch area; a waste holding area; and experimental procedure rooms.

2. A clean-dirty floor and work flow pattern that includes air locks and ultraviolet lights.

3. A specially designed, separate biohazardous study area including: shower-out capability, HEPA-filtered isolation cubicles, an autoclave, an ultrasound cleaner, a vertical laminar flow biological safety hood (Class II Type B) that is exhausted outside the work area through charcoal filters, a sink with bench surface, and a waste storage area.

4. Environmentally controlled features per room should include:

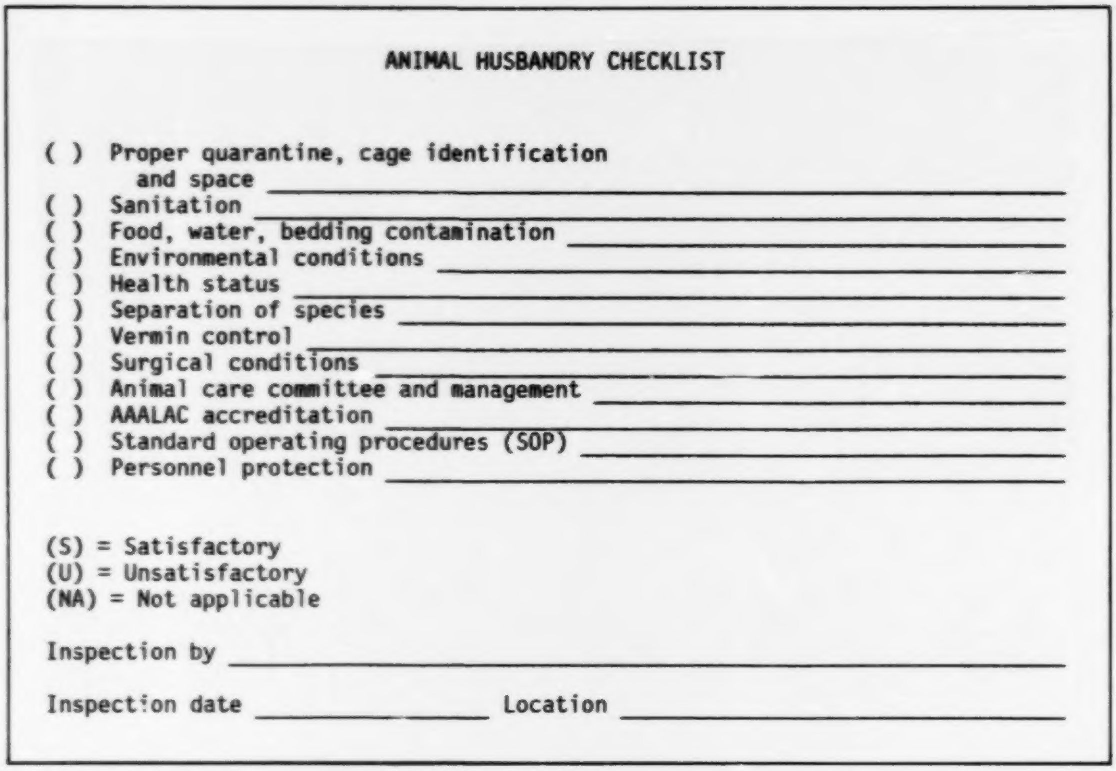
    a. Ventilation in animal rooms consisting of 100 percent fresh air with 10-15 changes per hour.

    b. Humidity controlled at 55 ± 5 percent (relative) with indicators displayed. Humidity should range between 40 to 70 percent.

    c. Temperature controlled at 72° ± 2° F (range between 64° and 80° F) with indicators displayed and temperature recorded by a high-low thermometer.

    d. Lighting controlled automatically (timers recessed into walls outside of room) at strengths of 75 to 100 footcandles. Light fixtures should be sealed against water and vermin.

    e. Dog kennels should be soundproofed, especially ceilings, ducts, and doors.



ANIMAL HUSBANDRY CHECKLIST

( ) Proper quarantine, cage identification
and space ______________________
( ) Sanitation ______________________
( ) Food, water, bedding contamination ______________________
( ) Environmental conditions ______________________
( ) Health status ______________________
( ) Separation of species ______________________
( ) Vermin control ______________________
( ) Surgical conditions ______________________
( ) Animal care committee and management ______________________
( ) AAALAC accreditation ______________________
( ) Standard operating procedures (SOP) ______________________
( ) Personnel protection ______________________

(S) = Satisfactory
(U) = Unsatisfactory
(NA) = Not applicable

Inspection by ______________________

Inspection date ____________ Location ______________________

Figure 7-2. Sample QC checklist for animal care. (Section 7.2.2 should be consulted for details.)

### INVESTIGATOR CHECKLIST FOR ANIMAL TESTING

( ) Background genetic information
( ) Transportation
( ) Environmental laboratory conditions
( ) Health status
( ) Sacrifice, surgery, quarantine
( ) Age
( ) Caging materials and environment
( ) Restraints, handling
( ) Nourishment
( ) Safety
( ) Drug route: Injection
Feed
( ) Toxic compounds
( ) Drug purity
( ) Drug stability
( ) Controls
( ) Drug interactions
( ) Recordkeeping
( ) Standard operating procedures (SOP)
( ) Biostatistics
( ) D.V.M./Pathologist
( ) Standard reporting forms

(S) = Satisfactory
(U) = Unsatisfactory
(NA) = Not applicable

Inspection by

Inspection date Location

Figure 7-3. Sample QC checklist for investigator using animals. (Section 7.3 should be consulted for details.)

f. Air pressure should be properly balanced and slightly negative to clean corridor but positive to soiled corridors.

5. Strategically located, separate quarantine and conditioning areas.

6. All selected materials utilized in the animal quarters should facilitate efficient and hygienic operation. Structural design should conform to all pertinent regulations. Structural materials and features are defined as follows:

   a. Corridors should be 7 ft wide with coved floor-wall junctions to facilitate cleaning. Curbs, guardrails, and bumpers should be installed for the protection of corners, doors, and wall-mounted devices.

   b. Animal room doors should open into rooms and measure 42 in. wide by 84 in. high. Each should feature a lock, window, inset handles, and an automatic closing device with a floor- or wall-mounted stop. Doors should seal when closed to prevent vermin and/or insect entrance. All doors should be metal and should feature kickplates.

   c. Floor surfacing should be barrier-sealed, smooth, nonabsorbant, nonslip, and wear-, acid-, and solvent-resistant. It should be monolithic with a minimal number of joints and should be able to withstand the wear and tear of racks, cages, and other heavy equipment.

   d. Walls should be well sealed, have smooth surfaces, and be resistant to detergents and other cleaning agents. They should be free of cracks, holes, and imperfect junctures.

   e. Ceilings should be smooth, waterproof, and free of penetrations and imperfect junctures.

   f. Floor drains may not be necessary, especially in rodent housing areas where wet/dry vacuum cleaning suffices. Floor drains ($\geq$4 in. diameter) should be provided in some rooms to offer flexibility. In areas of heavy usage, drains should measure $\geq$6 in. diameter with flush rims provided. Floor slope should be designed at 0.25 in./yd. The capability to cap and seal drains should be included. There should be no drains in surgery rooms.

### 7.2.2 Animal Husbandry Quality Control[13]

Appropriate animal husbandry quality control procedures should include but are not limited to:

1. Animal identification methods should be especially designed for the particular species.

2. Quarantine of animals should be consistently carried out in a separate area designed for this purpose. The procedures governing operations in this area should be strictly adhered to at all times.

3. Proper cage population densities should be maintained at all times. Refer to Table 7-1 for specific space recommendations.

4. Sanitization should be accomplished through the use of carefully selected products, schedules, and application patterns.

5. Animal caging should be sanitized by following a specific schedule for each species and project. The cage washers should be properly functioning at all times and records of internal temperature monitoring should be maintained.

6. Animal feeds should be appropriately selected, properly stored, and monitored for contamination and deterioration. See Table 7-2 for suggested maximum contamination levels. Table 7-3 illustrates QC tests done for HERL-RTP.

7. Fresh water should be available at all times (unless specifically denied); it should be potable and consistently monitored for contamination. Automatic systems should be carefully monitored for appropriate function and contamination.

8. Animal bedding should be appropriately selected, stored, and monitored for contamination.

9. Vermin control program should be carefully designed, applied, and monitored. Consistent records should be kept and descriptive information concerning the agents used should be available at all times.

10. Surgical procedures should be performed by qualified personnel only, humane methods should be strictly adhered to, thorough records maintained, appropriate preoperative protocols filed, and related equipment properly maintained.

11. Personnel should have appropriate training and experience and their performance should be routinely evaluated.

12. Standard operating procedures should be thoroughly spelled out, understood, and consistently applied in all areas.[13]

13. The Animal Care Advisory Committee should meet regularly, the meeting minutes should be carefully recorded and promulgated, and the specific functions of the committee should be well understood by each member.

14. Isolation of diseased animals (if so indicated) should be done promptly and in an area strategically separated from the main housing area.

## TABLE 7-1. SPACE RECOMMENDATIONS FOR LABORATORY ANIMALS

| Animals | Weight | Type of Housing | Floor Area/ Animal | Height[a] |
|---|---|---|---|---|
| Mice | < 10 g | Cage | 39 cm$^2$ (6 in.$^2$) | 12.7 cm (5 in.) |
| | 10–15 g | Cage | 52 cm$^2$ (8 in.$^2$) | 12.7 cm (5 in.) |
| | 16–25 g | Cage | 77 cm$^2$ (12 in.$^2$) | 12.7 cm (5 in.) |
| | > 25 g | Cage | 97 cm$^2$ (15 in.$^2$) | 12.7 cm (5 in.) |
| Rats | < 100 g | Cage | 110 cm$^2$ (17 in.$^2$) | 17.8 cm (7 in.) |
| | 100–200 g | Cage | 148 cm$^2$ (23 in.$^2$) | 17.8 cm (7 in.) |
| | 201–300 g | Cage | 187 cm$^2$ (29 in.$^2$) | 17.8 cm (7 in.) |
| | > 300 g | Cage | 258 cm$^2$ (40 in.$^2$) | 17.8 cm (7 in.) |
| Hamsters | < 60 g | Cage | 64.5 cm$^2$ (10.0 in.$^2$) | 15.2 cm (6 in.) |
| | 60–80 g | Cage | 83.9 cm$^2$ (13.0 in.$^2$) | 15.2 cm (6 in.) |
| | 81–100 g | Cage | 103.2 cm$^2$ (16.0 in.$^2$) | 15.2 cm (6 in.) |
| | > 100 g | Cage | 122.6 cm$^2$ (19.0 in.$^2$) | 15.2 cm (6 in.) |
| Guinea pigs | ≤ 350 g | Cage | 277 cm$^2$ (43 in.$^2$) | 17.8 cm (7 in.) |
| | > 350 g | Cage | 652 cm$^2$ (101 in.$^2$) | 17.8 cm (7 in.) |
| Rabbits | < 2 kg | Cage | 0.14 m$^2$ (1.5 ft$^2$) | 35.6 cm (14 in.) |
| | 2–4 kg | Cage | 0.28 m$^2$ (3.0 ft$^2$) | 35.6 cm (14 in.) |
| | 4–6 kg | Cage | 0.37 m$^2$ (4.0 ft$^2$) | 35.6 cm (14 in.) |
| | > 6 kg | Cage | 0.46 m$^2$ (5.0 ft$^2$) | 35.6 cm (14 in.) |
| Cats | ≤ 4 kg | Cage | 0.28 m$^2$ (3.0 ft$^2$) | 61.0 cm (24 in.) |
| | > 4 kg | Cage | 0.37 m$^2$ (4.0 ft$^2$) | 61.0 cm (24 in.) |
| Dogs[b] | < 15 kg | Pen or run | 0.74 m$^2$ (8.0 ft$^2$) | — |
| | 15–30 kg | Pen or run | 1.11 m$^2$ (12.1 ft$^2$) | — |
| | > 30 kg | Pen or run | 2.23 m$^2$ (24.0 ft$^2$) | — |
| | < 15 kg | Cage | 0.74 m$^2$ (8.0 ft$^2$) | 81.3 cm (32 in.) |
| | 15–30 kg | Cage | 1.11 m$^2$ (12.1 ft$^2$) | 91.4 cm (36 in.) |
| | > 30 kg | Cage | [b] | [b] |
| Primates[c] | | | | |
| Group 1 | ≤ 1 kg | Cage | 0.15 m$^2$ (1.6 ft$^2$) | 50.8 cm (20 in.) |
| Group 2 | ≤ 3 kg | Cage | 0.28 m$^2$ (3.0 ft$^2$) | 76.2 cm (30 in.) |
| Group 3 | ≤ 15 kg | Cage | 0.40 m$^2$ (4.3 ft$^2$) | 76.2 cm (30 in.) |
| Group 4 | > 15 kg | Cage | 0.74 m$^2$ (8.0 ft$^2$) | 91.4 cm (36 in.) |
| Group 5 | > 25 kg | Cage | 2.32 m$^2$ (25.1 ft$^2$) | 213.4 cm (84 in.) |
| Pigeons | — | Cage | 1451.7 cm$^2$ (115 in.$^2$) | [d] |
| Coturnix quail | — | Cage | 232.3 cm$^2$ (36 in.$^2$) | [d] |
| Chickens | < 0.5 kg | Cage | 232.3 cm$^2$ (36 in.$^2$) | [d] |
| | 0.5–2 kg | Cage | 464.5 cm$^2$ (72 in.$^2$) | [d] |
| | 2–4 kg | Cage | 1090.4 cm$^2$ (169 in.$^2$) | [d] |
| | > 4 kg | Cage | 1651.7 cm$^2$ (256 in.$^2$) | [d] |
| Sheep and goats | < 25 kg | Pen | 0.93 m$^2$ (10 ft$^2$) | — |
| | 25–50 kg | Pen | 1.39 m$^2$ (15 ft$^2$) | — |
| | > 50 kg | Pen | 1.86 m$^2$ (20 ft$^2$) | — |

(continued)

## TABLE 7-1 (continued)

| Animals | Weight | Type of Housing | Floor Area/ Animal | Height[a] |
|---|---|---|---|---|
| Swine | < 50 kg | Pen | 0.56 m² (6 ft²) | — |
| | 50–100 kg | Pen | 1.11 m² (12 ft | |
| | > 100 kg | Pen | 2.79 m² (30 ft²) | — |
| Cattle | < 350 kg | Stanchion | 1.49 m² (16 ft²) | — |
| | 350–450 kg | Stanchion | 1.77 m² (18 ft²) | — |
| | 451–550 kg | Stanchion | 1.95 m² (22 ft²) | — |
| | 551–650 kg | Stanchion | 2.23 m² (24 ft²) | — |
| | > 650 kg | Stanchion | 2.51 m² (27 ft²) | — |
| | < 75 kg | Pen | 2.23 m² (24 ft²) | — |
| | 75–200 kg | Pen | 4.64 m² (51 ft²) | — |
| | 201–500 kg | Pen | 9.29 m² (100 ft²) | — |
| | 501–600 kg | Pen | 11.15 m² (121 ft²) | — |
| | 601–700 kg | Pen | 13.01 m² (140 ft²) | — |
| | > 700 kg | Pen | 13.94 m² (151 ft²) | — |
| Horses | — | Tie stall | 4.09 m² (44 ft²) | — |
| | — | Pen | 13.38 m² (144 ft²) | — |

[a] From the resting floor to the cage top.

[b] These recommendations may require modifications according to the body conformation of particular breeds. As a further general guide, the height of a dog cage should be equal to the height of the dog over the shoulders (at the withers) plus at least 6 in. (15.2 cm), and the width and depth of the cage should each be equal to the length of the dog from the tip of the nose to the base of the tail plus at least 6 in. (15.2 cm).

[c] The primates are grouped according to approximate size. Examples of species that may be included in the various groups are:

Group 1—marmosets, tupaias, and infants of various species
Group 2—cebus and similar species
Group 3—macaques and large African species
Group 4—baboons, and nonbrachiating monkeys larger than 15 kg
Group 5—great apes and brachiating species

If primates are housed in groups in pens, only compatible animals should be kept. The minimal height of pens should be 6 ft (1.83 m). Resting perches and appropriate shelter should also be provided. In all cages, the minimal cage height for chimpanzees and brachiating species (orangutans, gibbons, and spider and woolly monkeys) should be such that the animal can swing from the cage ceiling without having its feet touch the floor of the cage when fully extended.

[d] Sufficient headroom must be provided for birds to stand erect.

SOURCE: Guide for the Care and Use of Laboratory Animals, U.S. DHEW, Publication No. (NIH)78-23, 1978.

TABLE 7-2. MAXIMUM CONCENTRATIONS OF FEED CONTAMINANTS CONSIDERED ACCEPTABLE FOR NATURAL INGREDIENT RATIONS MANUFACTURED FOR USE AT THE NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH AT JEFFERSON, ARKANSAS

| Agents | Maximum Concentration |
|---|---|
| Cadmium | 0.05 μg/g |
| Selenium | 0.50 μg/g |
| Polychlorinated biphenyls | 0.50 μg/g |
| Total DDT (DDE, DDT, TDE) | 0.05 μg/g |
| Mercury | 0.05 μg/g |
| Arsenic | 0.25 μg/g |
| Lead | 1.00 μg/g |
| Dieldrin | 0.01 μg/g |
| Lindane | 0.01 μg/g |
| Heptachlor | 0.01 μg/g |
| Malathion | 0.50 μg/g |
| Estrogenic activity | 2.00 μg/kg |
| Total aflatoxins ($B_1$, $B_2$, $G_1$, $G_2$) | 1.00 μg/kg |

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## TABLE 7-3. MONTHLY QC/QA TESTS PERFORMED FOR HERL/LAS

1. Nasopharyngeal cultures for Mycoplasma

2. Pathogenic respiratory bacteria (e.g., Staphylococcus aureus, E. coli, Streptococcus viridans, Proteus mirabilis, and P. morgani).

3. Pseudomonas (detection) from animal drinking water, feces, lung washings, and feeder surfaces, utilizing Pseudosal agar.

4. Murine virus antibody determination in serum.

5. Endoparasite detection for Syphacia muris and S. obvelata, Eimeria sp., Aspicularis tetraptera, Heterakis spumosa, Hymenolepsis nana and H. diminuta (utilizing zinc sulfate flotation and anal tape methods).

6. Ectoparasites: Myocoptes musculinus, Myobia ratti and M. musculi, Demodex sp. Polyplax spinulosa and P. serrata, and Notoedres sp.

7. Feed (Wayne Rabbit Diet, Purina Monkey Chow, Wayne Guinea Pig Diet, Purina Laboratory Chow, Wayne Lab Blox) and bedding (heat-treated white pine shavings) analysis for:

   Aflatoxins

   Metals

   Arsenic
   Bismuth
   Cadmium
   Lead
   Magnesium
   Manganese
   Mercury

   Bacterial Contamination

   Standard Plate Count for:
     Coliforms (total and fecal)
     E. Coli
     M.B.A.S.
     Yeast and Mold
     Pseudomonas
     Salmonella
     Shigella

   Pesticides

   Alpha BHC
   Beta BHC
   Gamma BHC - Lindane
   Delta BHC
   Heptachlor
   Aldrin
   Heptachlor Epoxide
   DDE
   DDD
   DDT
   Mirex
   Methoxychlor
   Dieldrin
   Endrin
   Telodrin
   Estimated PCB's
   Ronnel
   Ethion
   Trithion
   Diazinon
   Methyl Parathion
   Ethyl Parathion
   Malathion

8. Bacterial colony counts of animal rooms sampled immediately upon completion of room sanitization.

9. Temperature and humidity are checked twice daily (a.m. and p.m.) in all rooms.

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SOURCE: Linko, R. Standard Operating Procedures, HERL/LAS ACC, Room P302 EPA, RTP NC, 27711, 1980.

Note: Monthly summary reports can be obtained from the EPA Animal Care Coordinator.

15. Adequate personnel protection against inhalation of dust particles and exposure to carcinogens/mutagens must be provided.
16. Animal colony facilities should be accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC), as proof of high quality animal care.

## 7.3 INVESTIGATOR QUALITY CONTROL

High quality data are obtained more consistently when the entire research team consists of knowledgeable and dedicated individuals. Listed below are important parameters that investigators should be familiar with and should incorporate into the planning of their experimental protocols.

### 7.3.1 Experimental Laboratory Environment

1. The genetic background of the test species should be thoroughly assessed before selection.
2. The health status of the test species should be thoroughly evaluated.
3. The investigator should be fully aware of the effects of improper transportation on the research animal between laboratories or between the original supplier and the animal facility.
4. The laboratory environment should be as similar as possible to the animal housing environment with respect to temperature, humidity, ventilation, lighting, noise, and other important features (see Table 7-4).
5. The particular cage environment should be as similar as possible to the animal housing cage environment with respect to population density and bedding materials.

TABLE 7-4. RECOMMENDED TEMPERATURE AND RELATIVE HUMIDITY FOR COMMON RODENTS

| Rodent | Temperature °C | Temperature °F | Relative humidity (%) |
|---|---|---|---|
| Mouse | 20-24 | 68-75 | 50-60 |
| Hamster | 20-24 | 68-75 | 40-55 |
| Rat | 18-24 | 65-75 | 45-55 |
| Guinea pig | 18-24 | 65-75 | 45-55 |

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6. Proper methods should be selected when sacrificing animals.

7. Proper methods of restraint should be uniformly used to minimize stress.

8. Cage population density should be carefully monitored and maintained using standard guidelines (Table 7-1).

9. Animal feed and water quality should be the same as that used in the animal housing area (see Tables 7-5, 7-6, and 7-7). These parameters should be monitored continuously and the findings documented and recorded in a bound notebook.

10. Proper procedures to avoid cross-contamination between species should be carefully adhered to in the research laboratory.

### 7.3.2 Experimental Compound Testing

1. Experimental compound testing procedures should be thoroughly understood and uniformly applied, using the following guidelines:

    a. Injectable routes of administration should be used whenever possible, as injection affords the most reliable method of test compound absorption.

    b. The investigator should be thoroughly aware of the positive and/or negative effects of administering test compounds in the feed or drinking water.

    c. The investigator should be thoroughly aware of the safety aspects of adding carcinogenic/mutagenic type compounds to the feed or drinking water.

    d. The investigator should be thoroughly aware of the safety aspects of using highly toxic and/or carcinogenic injectable compounds with respect to the disposition of all contaminated and/or waste products.

    e. The investigator should be thoroughly aware of the chemical and biological purity as well as impurity of the test compound before initiating the study.

    f. The investigator should be thoroughly aware of the chemical stability and concentration of the test compound before study initiation. Samples should be collected and properly stored for later reference.

    g. The investigator should thoroughly characterize the chemical and biological properties of all positive controls, including vehicle compounds, before initiating the study.

## TABLE 7-5. AVERAGE DAILY NUTRIENT REQUIREMENT IN PERCENTAGE OF WHOLE DIET[8]

| Nutrients | Species (Adult) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Monkey (Rhesus) | Dog | Cat | Rat | Mouse | Rabbit | Guinea pig | Hamster |
| Protein | 15-20% | 20-24% | 30-40% | 16-20% | 16-20% | 15% | 20% | 24% |
| Fats | 3-5% | 4-9% | 20-30% | 5.0% | 3-12% | 3-5% | 3-5% | 3.5% |
| Carbohydrates | 45-55% | 40-60% | 25-35% | 45-55% | 45-55% | 45-55% | 45-50% | 45-65% |
| Calcium | 0.86% | 1.1% | Required | 0.6% | 0.5% | Required | 1.2% | 0.6% |
| Phosphorous | 0.47% | 0.9% | " | 0.6% | 0.5% | " | 0.6% | 0.35% |
| Magnesium | 0.11% | 0.05% | " | 0.05% | 0.05% | 350 mg/kg-D | 0.34% | ? |
| Sodium chloride | 0.5% | 1.5% | " | 0.5% | 0.5-1.0% | 0.7% | Required | ? |
| Potassium | 0.56% | 0.9% | " | 0.2% | 0.2% | Required | 1.6% | ? |
| Iron | 144 ppm | 57 mg/kg-D | " | 25 mg/kg-D | 25 mg/kg-D | " | Required | ? |
| Copper | 12 ppm | 7 mg/kg-D | " | 5 mg/kg-D | 5 mg/kg-D | " | " | ? |
| Cobalt | 0.54 ppm | 2.5 mg/kg-D | " | Trace | Trace | " | " | ? |
| Iodine | 84 ppm | 1.5 mg/kg-D | " | Trace | Trace | " | " | ? |
| Manganese | 47 ppm | 5 mg/kg-D | " | 0.002% | 0.002% | 7 mg/kg-D | " | ? |
| Zinc | 20 ppm | 5 mg/kg-D | " | 0.005% | 0.005% | Required | " | ? |
| Vitamin A | Required | 1.5 mg/kg-D | 25,000 IU/kg-D | 12,000 IU/kg-D | 500 IU/kg-D | " | 12 mg/kg-D | 13,000 IU/kg-D |
| Thiamine ($B_1$) | 0.75 mg/kg-D | 7.3 mg/kg-D | 4 mg/kg-D | 4 mg/kg-D | 3 mg/kg-D | " | 16 mg/kg-D | 6 mg/kg-D |
| Riboflavin ($B_2$) | 0.75 mg/kg-D | 2.2 mg/kg-D | 4 mg/kg-D | 4 mg/kg-D | 4 mg/kg-D | None | 16 mg/kg-D | 6 mg/kg-D |
| Pantothenic acid ($B_3$) | Required | 2.3 mg/kg-D | 5 mg/kg-D | -- | -- | None | 20 mg/kg-D | 40 mg/kg-D |
| Niacin ($B_5$) | 38 mg/kg-D | 10.5 mg/kg-D | 40 mg/kg-D | 15 mg/kg-D | 30 mg/kg-D | 200 mg/kg-D | 100 mg/kg-D | Required |
| Pyridoxine ($B_6$) | 1.3 mg/kg-D | 9.7 mg/kg-D | 4 mg/kg-D | 0.4 mg/kg-D | 1 mg/kg-D | Required | 16 mg/kg-D | 6 mg/kg-D |
| Choline | ? | 1.21 g/kg-D | 3 g/kg-D | 1 g/kg-D | 1 g/kg-D | Required | 1.5 g/kg-D | ? |
| Cyanocobalamin ($B_{12}$) | 25 µg/kg-D | 0.03 mg/kg-D | Trace | 5 µg/kg-D | 5 µg/kg-D | None | None | None |
| Vitamin C | 25 mg/kg-D | None | None | None | None | None | 200 mg/kg-D | None |
| Vitamin D | Required | 6.6 µg/kg-D | 1,000 IU/kg-D | 300 IU/kg-D | 18 IU/kg-D | Required | None | None |
| Vitamin E | Required | 48 mg/kg-D | 136 IU/kg-D | 20 mg/kg-D | 40 IU/kg-D | 7 mg/kg-D | 60 mg/kg-D | 25 mg/kg-D |
| Vitamin K | ? | ? | ? | 0.1 mg/kg-D | 1 mg/kg-D | ? | 10 mg/kg-D | Required |
| Inositol | ? | ? | ? | 0.3 mg/kg-D | 10-100 mg/kg-D | ? | None | ? |
| Folic acid | 1.3 mg/kg-D | ? | None | ? | ? | Required | 10 mg/kg-D | None |
| Biotin | 250 µg/kg-D | ? | ? | 0.3 ppm | 0.34 ppm | Required | None | None |
| Calcium pantothenate ($B_3$) | -- | -- | -- | 10 mg/kg-D | 8.5 mg/kg-D | None | -- | -- |
| Para-aminobenzoic acid (PABA) | -- | ? | Required | -- | -- | ? | 100 mg/kg-D | ? |

NOTE: Fiber or roughage in standard commercial diets is about 10 percent for the guinea pig, 15 percent for the rabbit, 2.5 to 5.5 percent for the other common laboratory animals listed here.

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## TABLE 7-6. BIOLOGICALLY EFFECTIVE CONCENTRATIONS OF SELECTED HEAVY METALS

| | | | Dietary or water concentration (ppm) | |
|---|---|---|---|---|
| Element | Species | End point | Effect | No effect |
| As | Mouse | Reproduction | 5 | - |
| | | Growth and mortality | - | 5 |
| | | Mortality | 5 | - |
| | | Mammary tumors[a] | 10 | - |
| | Rat | Growth | 62.5[b] | 31.25[b] |
| Cd | Mouse | Reproduction | 10 | - |
| | | Mortality | 5 | - |
| | Rat | Renal vasculature | 0.2 | - |
| | | Hypertension | 1 | - |
| Pb | Mouse | Mortality | 5 | - |
| | | Growth | - | 5 |
| | | Antibody formation | 14 | - |
| | Rat | δ-Aminolevulinate dehydratase | 10 | 1 |
| | | Mortality | 5 | - |
| Ag | Mouse | Mortality and growth | 5 | - |
| | Rat | Hypertension | 5 | 2.5 |
| | | Behavior and learning | 2[b] | - |
| | | Kidney ultrastructure | 2[b] | - |
| Se | Mouse | Reproduction | 3 | - |
| | | Growth and mortality | 3 | - |
| | | Mammary tumors[a] | 2 | - |
| | Rat | Growth, mortality and tumors | 2 | - |
| | | AAF-induced tumors[d] | 0.5[b] | 0.1[b] |
| | | Hepatitis | 2.5[b] | 0.5[b] |

[a] Decreased incidence, increased tumor growth rate.

[b] Mixed in the diet, all others were administered in water.

[c] Decreased tumor incidence.

[d] Increased latent period.

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## TABLE 7-7. BIOLOGICALLY EFFECTIVE CONCENTRATIONS OF SELECTED ORGANIC FEED CONTAMINANTS

| | | | Dietary concentration (ppm) | |
|---|---|---|---|---|
| Compound | Species | End point | Effect | No effect |
| Aflatoxin ($B_1$) | Mouse | Liver lipids | 0.114 | 0.057 |
| | | Liver tumors | 1 | - |
| | Rat | Liver hyperplasia | 0.001 | - |
| | | Liver tumors | 0.015 | - |
| DDT | Mouse | Reproduction | - | 3 |
| | | Neoplasia | 3 | - |
| | | Liver tumors | 2 | - |
| | | Liver tumors | 250 | 20 |
| | | Lung tumors | 10 | - |
| | Rat | Liver morphology | 5 | 1 |
| | | Microsomal enzymes | 2.5 | 2 |
| | | Microsomal enzymes | 1 | 0.2 |
| Dieldrin | Mouse | Reproduction | 2.5 | - |
| | | Mortality | 10 | 1 |
| | | Liver tumors | 0.1 | - |
| | Rat | Microsomal enzymes | 5 | 1 |
| | | Microsomal enzymes | 5 | 2 |
| | | Liver weight | 1 | 0.1 |
| | | Brain lesions | 0.34 | 0.02 |
| Heptachlor | Mouse | Liver weight | 1 | - |
| | | Liver tumors | 5 | 1 |
| | Rat | Microsomal enzymes | 5 | 1 |
| | | Microsomal enzymes | 2 | - |
| Lindane | Mouse | Tumor induction and liver morphology | - | 50 |
| | Rat | Microsomal enzymes | 50 | 20 |
| | | Microsomal enzymes and liver weight | 20 | 2 |
| Malathion | Rat | Liver weight | 100 | - |
| | | Cholinesterase | 1,000 | 100 |
| | | Cholinesterase and EEG | 380[a] | - |
| | | DMBA-induced tumors | 250 | - |

[a] Estimated equivalent.

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h. The investigator should be thoroughly aware of the solubility parameters of the compounds involved as well as their possible interactions in animal systems and their expiration dates.

i. The investigator should monitor the stability of the tested compounds and maintain thorough records of the results.

### 7.3.3 Experimental Design and Data Reporting

1. All data, including experimental design, observations, and information on test animal physiological parameters, should be fully documented on standard forms or in a bound notebook, whichever is more appropriate.
2. Any changes or deviations from standard operating procedures (SOP) should be documented in writing after approval from the principal investigator.
3. A qualified biostatistician should review all research protocols to ensure proper implementation of statistical procedures.
4. A Doctor of Veterinary Medicine should be consulted for recommendations on experimental procedures and test animal health verification.

## 7.4 SUMMARY

The purpose of this section was to provide the staff and scientific investigators of HERL-RTP with basic information necessary to provide proper animal care. Following these quality control and protocol guidelines will increase sensitivity, quality, and reproducibility of results and the longevity of test animals.

A properly designed animal research program should address total data quality, including quality control and quality assurance activities. The major effort in quality control is made by EPA/HERL-RTP, the operating organization. Consequently, the principal investigator and the laboratory animal staff are responsible for quality control. Internal quality assurance is the responsibility of the animal care coordinator and the animal care committee. External quality assurance is provided by outside audits conducted by organizations such as AAALAC ensure that proper animal handling techniques are being followed. These external audits are encouraged because they will assist in the objective definition of problem areas and reward and document HERL-RTP's ability and commitment to obtaining animal data of the highest quality. Recent provisional accreditation of the HERL-RTP animal facilities by AAALAC has verified that animal care is acceptable.

In summary, quality control is consistent with the implementation of good scientific methodology and with the systematic attention to eliminating the causes of poor data quality.

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# APPENDIX A

## CHEMICAL PRODUCTS USED IN THE HERL-RTP ANIMAL CARE FACILITIES

### CHEMICAL PRODUCTS USED IN THE EPA ANIMAL CARE FACILITIES*

I. Approved Disinfectants

A. Nolvasan-S, Chlorhexidine based virucide, Fort Dodge Laboratories, Inc.

Active ingredient:

1,1'-Hexamethyl(exebis)-[5-(p-chlorophenyl) biguanide] diacetate--2%

Concentration: 3 ounces Nolvasan-S to 1 gallon water.

B. T.B.Q. Germicidal Detergent, quaternary ammonium-based germicide, Vestal Laboratories.

Active ingredients:

N,N-bis [2-(omega-hydroxpoly(oxyethylene)) ethyl] alkylamine--12%
Alkyl (50% $C_{14}$, 40% $C_{12}$, 10% $C_{16}$) dimethyl benzyl ammonium chloride--(trade name: Zephiran chloride) 8%

Concentration: 2 ounces T.B.Q. to 1 gallon water.

C. BGC-3 broad-spectrum germicidal cleaner, multiphenolic based germicide, DuBois Chemicals.

Active ingredients:

Sodium lauryl ether sulfate--99%
Sodium ortho-phenylphenate--8.85%
Sodium ortho-benzyl-para-chlorophenate--7.92%
Isopropyl alcohol--7.39%
Sodium para-tertiary-amylphenate--4.10%
Tetrasodium ethylenediamine tetracetate--0.95%

Concentration: 0.5 (1/2) ounce BGC-3 to 1 gallon water.

II. Detergents

A. Life Sci 100, alkaline ware washing agent, DuBois Chemicals.

Ready-mixed concentration provided.

B. Life Sci 200, concentrated liquid phosphoric acid cleaner, DuBois Chemicals.

Ready-mixed concentration provided.

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*Source: R. Linko, Standard Operating Procedures, HERL/LAS ACC, Room P302, No. 247, Environmental Protection Agency, Research Triangle Park, NC, 1980.

C. Septisol Solution (Hexachlorophene), health care personnel hand wash.

Ready-mixed concentration provided.

D. Du-DRI, concentrated, acidic, liquid rinse additive, DuBois Chemicals.

Ready-mixed concentration provided.

III. Odor Counteractants

A. Arrest, water-based maskant, DuBois Chemicals.

Concentration: 2 ounces Arrest to 1 gallon water.

IV. Insecticides

A. Baygon 1.5, emulsifiable insecticide, Mobay Chemical Corporation.

Active ingredients:

2-(1-Methylethoxy) phenol methylcarbamate--13.9%

Concentration: 8 ounces Baygon 1.5 to 1 gallon water.

B. Diazinon-4E, insecticide, Stephenson Chemical Company, Inc.

Active ingredients:

0,0-diethyl 0-(2-isopropyl-6-methyl-4-pryimidinyl)
Phosphorothioate--47.55%
Tetrachloroethylene--8.70%
Aliphatic petroleum distillates--26.15%

C. Drione, insecticide, FMC Corporation.

Active ingredients:

Pyrethrins--1%
Piperonyl butoxide, technical--10%
Amorphous silica gel--40%
Petroleum hydrocarbons--49%

Concentration: This insecticide is applied as a powder as packaged.

V. Miscellaneous.

A. Chlorox
B. Comet
C. Lysol

# APPENDIX B

## ZOONOSES--EXPERIMENTAL ANIMALS TO MAN

### A: BACTERIAL DISEASES: —

| Disease In Man | Causative Agent | Common Vertebrate Hosts* | Means of Spread | Vectors and Notes on Cycle |
|---|---|---|---|---|
| 9. Pseudotuberculosis | P.pseudotuberculosis and some Fungi | Rodents, Pigeons, Turkeys<br>Canaries & misc. wild birds | Contact<br>Ingestion | — |
| 10. Rat Bite Fever | S.moniliformis<br>Spirillam minus | Rodents<br>Rodents | Rodent bites, Ingestion<br>Rodent bites | — |
| 11. Salmonellosis | Salmonella spp. | Farm animals, Rodents<br>Reptiles, Amphibia | Ingestion; Inhalation<br>Contact | — |
| 12. Shigellosis Bacillary Dysentry | Shigella spp. | Non-human primates | Contact, fecal contamination | — |
| 13. Tetanus | Cl.tetani | Horse and other Equidae | Contaminated puncture wounds | Soil, many mammalian spp. |
| 14. Tuberculosis | M. tuberculosis<br>M.bovis<br>M. avium | Primates, Cattle, Dogs<br>Cattle, Dogs<br>Poultry, Swine, Sheep | Contact, Ingestion Inhalation, Needles — hypodermic & tattoo | Anthropozoonotic** |
| 15. Tularemia | F. tularensis | Rabbits, Wild rodents<br>Sheep | Contact<br>Ingestion | Biting insects and ticks |
| 16. Vibriosis | Vibrio fetus | Domestic ruminants | Unknown (contact?) | — |

* Only more common host species are listed.

** Man is the primary vertebrate host.

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## APPENDIX B
## ZOONOSES--EXPERIMENTAL ANIMALS TO MAN (cont'd)

### B. RICKETTSIAL - DISEASES:

| Causative Agent | Diseases In Man | Common Vertebrate Hosts | Means of Spread; Vectors, Cycle Notes |
|---|---|---|---|
| R. akari | Rickettsial Pox | Mice | Mite bites; Allodermanyssus sanguineus |
| R. rickettsia | Rocky Mountain Spotted Fever | Rodents, Rabbits<br>Young sheep & dogs | Tick bites; Dermacentor spp;<br>American dog tick |
| R.australis<br>R.sibiricus | Queensland, North Asian Tick Typhus etc. of Eastern/Asiatic Countries | Various Mammalian Tick-host species | Tick bites; ticks themselves may act as reservoirs with tick to tick passage |
| R. mosseri | Murine Typhus | Rats; Mice | Flea bites from rat fleas; Rat to rat spread by lice also |
| Coxiella burnetti | Q fever | Cattle; Sheep | Inhalation; No overt disease in natural hosts; virus concentrated in placenta |

### C. ARBOVIRUS DISEASES:

| | | | |
|---|---|---|---|
| E.E.E. virus | Eastern Equine Encephalomyelitis | Birds; Horses | Mosquito bites; Bird/Mosquito/ Horse natural cycle |
| W.E.E. virus | Western Equine Enceph | Birds; Horses | " " " " |
| V.E.E. | Venezuelan Equine Encephalitis | Horses | Natural cycle Horse/Mosquito only |
| S.L.E | St. Louis Encephalitis | Birds | Natural cycle Bird/Mosquito only |
| Powassan virus | Powassan Encephalitis | Rodents (?) | Tick bites; |
| Colorado tick — borne virus | Colorado tick fever | Ground squirrels; Peromyscus spp. | Tick bite; Tick/small Rodent natural cycle |
| Various Asian Arboviruses | Various tick-borne Hemorrhagic Fevers | Rodents; Hares; Monkeys | Tick bites; Sub tropical climate conditions favor cycle. |

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# APPENDIX B
# ZOONOSES--EXPERIMENTAL ANIMALS TO MAN (cont'd)

## D. OTHER VIRUS DISEASES:

| Causative Agent | Diseases in Man | Common Vertebrate Hosts | Means of Spread, Vectors, Cycle Notes |
|---|---|---|---|
| Hemorhagic fever virses | S. American and Korean Hemorhagic fever. Lassa fever | Wild rodents<br>Mastomys ratalensis | Contact; Contamination of food etc. with rodent excreta. Direct contact |
| L.C.M. virus | Lymphocytic Chorio — Meningitis | Rodents; numerous other mammals | Contact; Inhalation; Congenital transmission; Tissue culture transmission. |
| Herpes simiae | Herpes B.Encephalitis | Rhesus; Other Macaca | Contact; bite wounds; Old World monkeys |
| Hepatitis virus | Hepatitis A. | Chimpanzees; | Contact; Anthropozoonotic diseases*. |
| Rabies virus | Rabies | Dogs, Bats & many others | Bites; Saliva contact; virus conc. in salivia. |

* Man is primary host. Measles (Rubeola) is another anthropozoonotic virus to non-human primates.

## E. FUNGAL and PROTOZOAN DISEASES:

| Causative Agent | Diseases in Man | Common Vertebrate Hosts | Means of Spread, Vectors, Cycle Notes |
|---|---|---|---|
| Trychophyton spp.<br>Microsporum spp.<br>Other dermatophytes | Ringworm dermatomycoses | Dog, Cat, G. Pig<br>Rodents and Farm animals | Direct contact, Ringworm of man can be transmitted to animals and visa-veras. Soil may be reservoir. |
| Histoplasma capsulatum | Histoplasmosis | Dogs, other domestic and wild species | Inhalation of fungi. May also grow in soil. |
| Coccidioides immitis | Coccidioidomycosis | Cattle, Dogs and occassionally other spp. | Inhalation of air-borne spores<br>Fungus present in desert soil. |
| Toxoplasma gondii | Toxoplasmosis | Cats; occassionally other other domestic & lab spp. | Ingestion of oocyots from cats. Inhalation Infected meat; Fetal transmission may occur. |
| Trypanasoma spp.<br>Plasmodiuma spp.<br>Leishmania spp. | Blood protozoan diseases | Non human Primates, Rodents Domestic and wild spp. | Insect vectors — saliva transmission. Some few species direct transmission. |
| Entamoeba histolytica | Amebiasis<br>Amebic dysentery | Dogs, Non human Primates | Contamination of food, usually by man (natural host) to dogs. |

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# APPENDIX C
# PHYSIOLOGICAL AND NUTRITIONAL PARAMETERS

| Species (age & weight) | Rectal temp. (° C ± 0.5) | Resp. rate (minimum X̄ & range) | Heart rate (minimum X̄ & range) | Water required (daily) | Urine excreted (daily) | Food required (daily) | Digestible protein % |
|---|---|---|---|---|---|---|---|
| Monkey<br>(M. fascicularis) | 39.0 | 40<br>30-54 | 220<br>165-243 | 350-950 mL | 150-550 mL | 350-550 g | 17 |
| Mouse | 37.5 | 138<br>90-180 | 470<br>300-650 | 3-7 mL | 1-3 mL | 3-6 g | 12 |
| Guinea pig | 39.5 | 86<br>60-110 | 280<br>250-300 | 12-15 mL/<br>100 g body wt. | 15-75 mL | 20-35 g<br>+ Vit.C. supp. | 25-30 |
| Hamster | 39.0 | 77<br>40-120 | 332<br>286-400 | 8-12 mL | 6-12 mL | 7-15 g | 16 |
| Rabbit | 39.5 | 40<br>35-56 | 260<br>205-308 | 80-100 mL/<br>kg body wt. | 50-90 mL/<br>kg body wt. | 75-100 g | 14 |
| Rat | 37.5 | 92<br>80-150 | 350<br>260-450 | 20-45 mL | 10-15 mL | 10-20 g | 12 |
| Baboon | 39.0 | 25<br>22-35 | 115<br>105-150 | 400-600 mL | 150-400 mL | 1-1.5 kg | 17 |
| Cat | 38.5 | 26<br>20-30 | 150<br>110-226 | 150 mL<br>100-200 | 50-120 mL | 110-225 g | 30 |

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# APPENDIX D
## CLINICAL CHEMISTRY REFERENCE VALUES

| Species | Sodium (mEg/L) | Potassium (mEg/L) | Chloride (mEg/L) | Bicarbonate (mgEg/L) | Inorganic phosphorus (mg/dL) | Calcium (mg/dL) | Magnesium (mg/dL) |
|---|---|---|---|---|---|---|---|
| Monkey (M. fascicularis) | 146-152 | 4-5 | 101-108 | 30<br>19-35 | 5-5.4 | 9.5-10.7 | - |
| Mouse | 136<br>128-186 | 5.3<br>4.9-5.9 | 108<br>105-110 | 25.5<br>20-32 | 6.0<br>2.3-9.2 | 6.4<br>3.2-8.5 | 2.3<br>0.8-3.9 |
| Rabbit | 144<br>138-160 | 6.0<br>3.7-6.8 | 103<br>92-112 | 24<br>16-32 | 4.9<br>2.3-6.9 | 9.9<br>5.6-12.7 | 2.8<br>2-5.4 |
| Rat | 147<br>140-156 | 6.2<br>5.4-7.0 | 102<br>100-110 | 22<br>13-3.2 | 7.9<br>3-11 | 11.5<br>5-14 | 2.9<br>1.6-4.4 |
| Guinea pig | 123<br>120-149 | 5.0<br>3.8-7.9 | 94<br>90-115 | 21.5<br>13-30 | 5.3<br>3-7.6 | 10.2<br>5.3-12 | 2.4<br>1.8-3.0 |
| Hamster | 131<br>106-146 | 5.0<br>4.0-5.9 | 95<br>86-112 | 38.2<br>33-44 | 5.7<br>3.4-8.2 | 9.9<br>5-12 | 2.4<br>1.9-3.5 |

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## APPENDIX D (continued)
## CLINICAL CHEMISTRY REFERENCE VALUES

| Species<br>$\bar{X}$ range | Glucose (Md/dL) | B.U.N.[a] (mg/dL) | Cholesterol (mg/dL) | Total protein (g/dL) | Albumin (g/dL) | S.G.O.T.[b] (I.V./L) | S.G.P.T.[c] (I.V./L) | Alkaline phosph. (I.V./L) |
|---|---|---|---|---|---|---|---|---|
| Monkey (*M. fascicularis*) | 60-90 | 18-28 | 100-150 | 7.5-8.7 | 2.4-3.4 | 34-56 | 21-39 | 15-35 |
| Mouse | 89<br>63-176 | 19.5<br>14-28 | 64<br>26-82 | 6.2<br>4-8.6 | 3<br>2.5-4.8 | 36<br>23-48 | 13<br>2-24 | 19<br>10-28 |
| Rabbit | 132<br>78-155 | 18.5<br>9-32 | 26<br>20-83 | 6.8<br>5-8 | 3.3<br>2.5-4 | 71<br>42-98 | 65<br>49-79 | 130<br>90-170 |
| Rat | 75<br>50-135 | 14.5<br>5-29 | 27<br>10-54 | 7.6<br>4.7-8.2 | 3.7<br>2.7-5.1 | 63<br>46-81 | 24<br>18-30 | 87<br>57-128 |
| Guinea pig | 92<br>82-107 | 23.5<br>9-32 | 30<br>16-43 | 5.2<br>5-6.8 | 2.6<br>2.1-3.9 | 47<br>27-68 | 42<br>25-59 | 70<br>55-108 |
| Hamster | 69<br>33-118 | 22<br>12-26 | 53<br>10-80 | 7.1<br>4-8 | 3.3<br>2.5-4 | 100<br>38-168 | 24<br>12-36 | 17<br>3-31 |

[a] Blood urea nitrogen.

[b] Serum glutamic oxalacetic transaminase.

[c] Serum glutamic pyruvic transaminase.

# APPENDIX E
# HEMATOLOGY
## Mean Values and Ranges

| Species (age & weight) | RBC ($\times 10^6/\text{mm}^3$) | Hb. (g/100 mL) | PCV (mL %) | Platelets ($\times 10^3/\text{mm}^3$) | WBC ($\times 10^3/\text{mm}^3$) | Neotrophils (%) | Lymphocytes (%) | Blood vol. (mL/kg) |
|---|---|---|---|---|---|---|---|---|
| Monkey<br>(M. fascicularis) | 5<br>4-6 | 10-12 | 35-43 | 300-500 | 5-10 | 50<br>30-65 | 45<br>25-70 | 55-75 |
| Mouse | 9.2<br>7-13 | 11.1<br>10-14 | 41.8<br>33-50 | 240<br>150-400 | 13.6<br>6-17 | 17.2<br>12-25 | 72.3<br>65-85 | 70-80 |
| Baboon | 5<br>4-6 | 12<br>8-16 | 35.6<br>30-43 | 100-450 | 5-17 | 27-73 | 26-59 | 50-70 |
| Cat | 7.3<br>5-10 | 10.5<br>8-15 | 40.5<br>24-45 | 228<br>100-700 | 17.0<br>5-20 | 57.1<br>35-75 | 32.2<br>20-55 | 45-75 |
| Rabbit | 6.5<br>5-8 | 13.5<br>8-17 | 40.8<br>31-50 | 468<br>250-750 | 8.6<br>3.0-12.5 | 45.0<br>30-65 | 40.1<br>28-85 | 45-70 |
| Rat | 8.5<br>6-10 | 14.2<br>11-17 | 45.9<br>40-50 | 330<br>150-460 | 9.8<br>5-13 | 25.5<br>5-49 | 74.0<br>43-85 | 50-65 |
| Guinea pig | 5.2<br>3-7 | 14.3<br>11-17 | 43.6<br>37-50 | 477<br>250-750 | 11.2<br>6-17 | 37.0<br>20-56 | 55.7<br>40-80 | 65-90 |
| Hamster | 7.2<br>4-10 | 16.4<br>13-19 | 50.8<br>39-59 | 386<br>300-570 | 8.1<br>5-11 | 25.5<br>15-35 | 70.8<br>55-92 | 65-80 |

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